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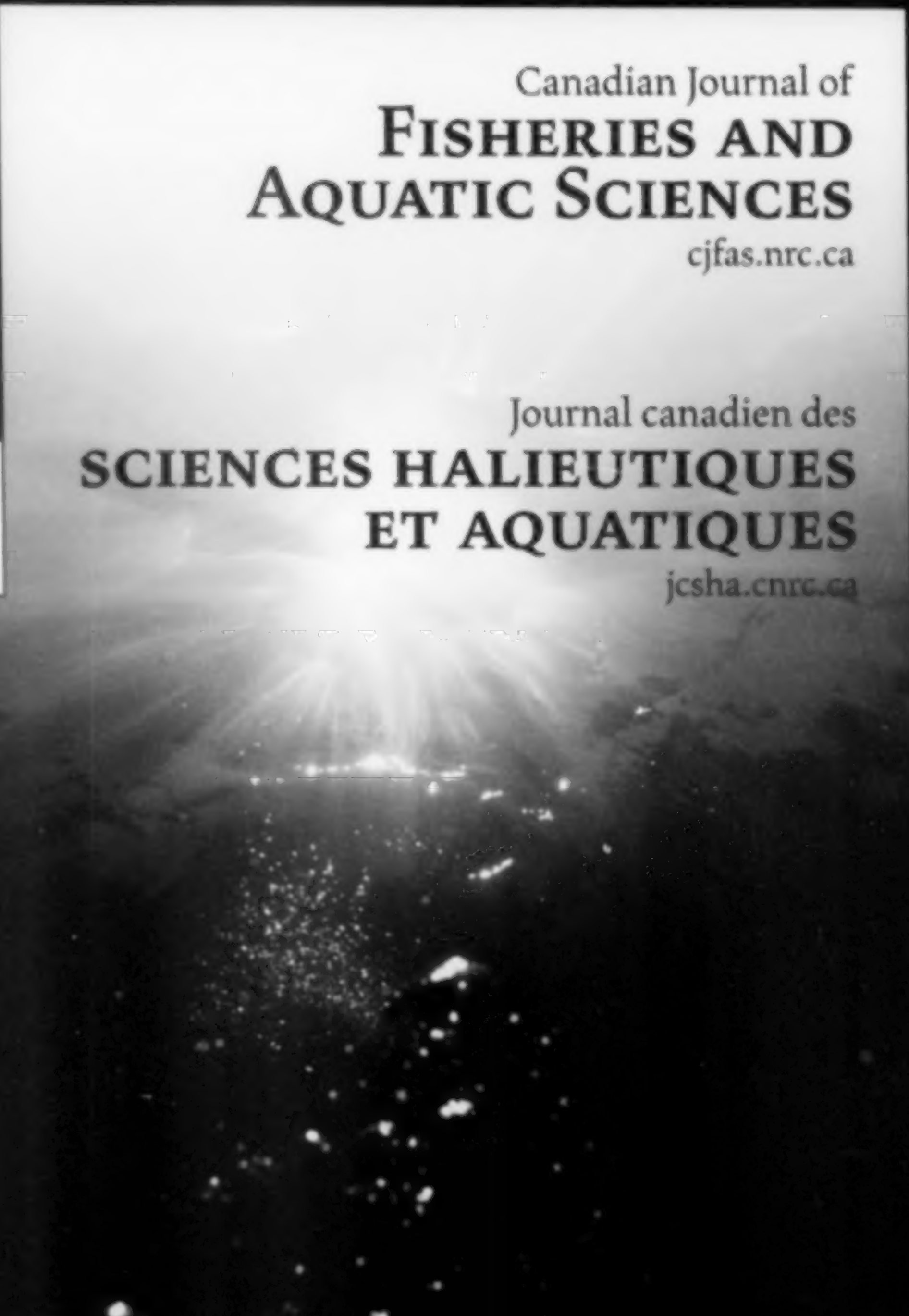

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Le projet « Forest Watershed and Riparian Disturbance » (FORWARD) a été lancé en 2001. L'objectif général du projet FORWARD est l'étude, sur le terrain, de la végétation, des sols, des conditions climatiques ainsi que de l'écoulement et de la qualité des eaux de surface sur les bassins-versants de la forêt boréale soumis aux effets de l'exploitation forestière et des perturbations causées par le feu. Ces travaux permettent de compiler une base de données détaillée sur les états antérieurs et postérieurs aux perturbations qui donne la possibilité d'effectuer des analyses scientifiques et facilite le développement d'outils de modélisation de l'écoulement et de la qualité des eaux. Les résultats de recherche et les outils de modélisation sont destinés à aider le secteur forestier à planifier l'aménagement et l'exploitation de la forêt en tenant compte des effets potentiels de ses activités sur l'écoulement et la qualité des eaux dans le cadre d'une approche fondée sur l'étude des bassins-versants. Les articles qui constituent le présent numéro supplémentaire de la *Revue du génie et de la science de l'environnement* décrivent les résultats initiaux de plusieurs projets de recherche et de modélisation, dont l'exécution se poursuit dans le cadre du projet FORWARD.

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RAPID COMMUNICATION / COMMUNICATION RAPIDE

Bacteria associated with the mucus layer of *Merlangius merlangus* (whiting) as biological tags to determine harvest location

Cindy J. Smith, Bret S. Danilowicz, and Wim G. Meijer

Abstract: We describe a new technique to determine the harvest location of *Merlangius merlangus* (whiting) based on exploiting the natural bacterial populations associated with the mucus layer of fish and surrounding seawater as biological tags. Bacterial community profiles from the outer and mouth mucus and the surrounding seawater were characterized by terminal restriction fragment length polymorphism (T-RFLP), and fish harvest location was predicted based on T-RFLP profile comparisons between fish and water and between individual fish. Fish harvest location was not resolved reliably based on analysis of the shared seawater and outer-mucus markers. However, comparisons based on shared seawater and mouth-mucus markers placed fish within 1.92 (± standard error (SE) of 0.31) stations ($p < 0.002$), equivalent to 153.0 km, of their known harvest location. Fish placement resolution was further increased when fish-to-fish bacterial assemblage comparisons were made. Based on outer-mucus, mouth-mucus, and combined outer- and mouth-mucus analysis, fish were placed within 1.00 (±SE 0.27), 1.55 (±SE 0.28), and 0.71 (±SE 0.24) ($p < 0.04$) stations, equivalent to 79.7, 123.5, and 57.3 km, respectively, of their known harvest location.

Résumé : Nous décrivons une nouvelle technique pour déterminer le lieu de récolte de *Merlangius merlangus* (merlus) basée sur l'utilisation des peuplements naturels de bactéries associés à la couche de mucus des poissons et à l'eau de mer environnante comme étiquettes biologiques. Nous avons caractérisé les profils des communautés bactériennes du mucus externe et buccal ainsi que de l'eau de mer environnante à l'aide du polymorphisme de la longueur des fragments de restriction terminaux (T-RFLP); nous avons ensuite prédit le lieu de capture d'après la comparaison des profils T-RFLP entre les poissons et l'eau et entre les poissons individuels. Il n'est pas possible de déterminer de manière fiable le lieu de récolte des poissons à partir de l'analyse des marqueurs communs de l'eau de mer et du mucus externe. Cependant, les comparaisons basées sur les marqueurs communs de l'eau de mer et du mucus oral permettent de placer les poissons à moins de 1,92 (± écart type (ET) de 0,31) station ($p < 0{,}0002$), soit l'équivalent de 153,0 km, de leur point connu de récolte. Une comparaison des communautés bactériennes entre les poissons accroît davantage la résolution de l'identification des points de capture. Selon que l'analyse est basée sur le mucus externe, le mucus oral, ou une combinaison de mucus externe et oral, les poissons sont assignés à respectivement moins de 1,00 (±ET 0,27), 1,55 (±ET 0,28) et 0,71 (±ET 0,24) ($p < 0{,}04$) stations, soit l'équivalent de 79,7, 123,5 et 57,3 km, de leur point connu de récolte.

[Traduit par la Rédaction]

Overexploitation of commercial fish stocks is a global problem, as many fish are being harvested below biologically safe limits. Improved techniques to determine fish harvest location are therefore needed to protect areas that are temporarily or permanently closed from fishing. Currently, a number of different methods based on variables such as parasite assemblages (MacKenzie 2002), parasite genetic markers (Cross et al. 2007), fish body morphology (Elliott et al. 1995), fish genetic markers (Shaklee et al. 1999), and otolith microchemical composition (Brophy and Danilowicz

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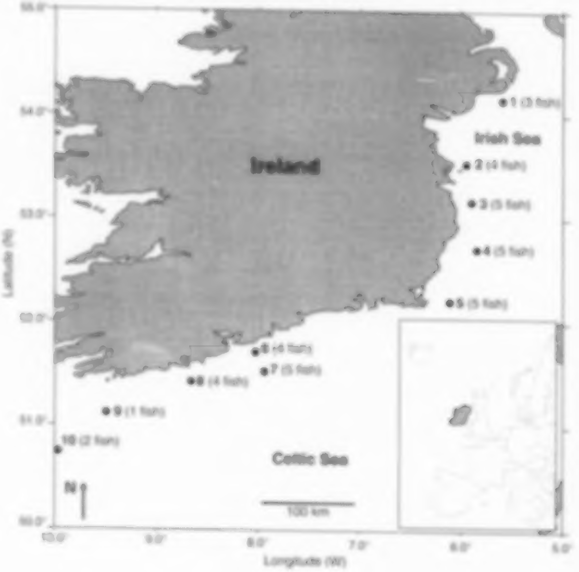
2002) and (or) otolith morphometry (Thorrold et al. 1998) are used to place fish to geographical locations. However, these studies generally focus on other aspects of fisheries management such as stock identification, migration patterns, or spawning locations as opposed to determining harvest location. A reliable method to determine fish harvest location is highly desirable for fisheries management, food traceability, and consumer confidence to ensure that fish are sourced from sustainable managed fish stocks.

In this study, we present the potential use of natural bacterial assemblages associated with the mucus layer of marine fish and those present in the surrounding seawater as novel biological tags to determine fish harvest location. Using established techniques from the field of environmental microbiology (genomic DNA extraction, polymerase chain reaction (PCR) amplification of the 16S rRNA gene from environmental samples, and subsequent bacterial community "fingerprinting" using terminal restriction fragment length polymorphism (T-RFLP); Avaniss-Aghajani et al. 1994), we describe a novel method to determine fish harvest location. T-RFLP profiles (bacterial community "fingerprints") generated from the fish mucus layer of whiting and surrounding seawater samples were compared to determine if fish harvest location could be reliably resolved based (*i*) on comparisons of shared fish mucus layer and water bacterial assemblages and (*ii*) on comparisons of bacterial assemblages of the mucus layers of individual fish.

Water samples (7 L collected 15 m from the seabed) and whiting (*Merlangius merlangus*; $n = 39$, harvested by bottom trawling for 30 min) were sampled from 10 stations in the Irish and Celtic seas (Fig. 1) as described previously (Smith et al. 2005, 2007). Mucus samples were taken aseptically from the outer mucus and mouth mucus of individual fish and snap-frozen for subsequent molecular analysis. Nucleic acids were extracted from frozen samples (fish mucus and water) using a modified version of the QIAamp mini DNA extraction kit (Qiagen, Crawley, UK) (Smith et al. 2005, 2007). 16S rRNA genes were amplified in duplicate from each environmental DNA extract, and PCR products were subjected to single digestions with restriction enzymes *Cfo*I, *Msp*I, and *Rsa*I (Roche, Sussex, UK) for subsequent T-RFLP analysis using a Beckman Coulter CEQ 2000 XL DNA analysis system as outlined in Smith et al. (2005, 2007). Individual-sample duplicate T-RFLP profiles were aligned to produce a single reliable T-RFLP profile using T-Align (Smith et al. 2005), thus only terminal restriction fragments (TRFs) present in both digests were used in the subsequent analysis. These single T-RFLP profiles generated from both fish and water stations were aligned, using T-Align, to identify all shared and unique TRFs.

In the first test, fish harvest location prediction based on shared water and mucus TRFs was investigated. The T-RFLP profiles derived from the outer- and mouth-mucus layers were compared separately. Water and outer- or mouth-mucus T-RFLPs were aligned, and TRFs present in both the water and mucus layer were identified and used for further analysis. The data were converted to presence or absence, and a Jaccard similarity matrix (SPSS ver. 14.0, SPSS Inc., Chicago, Illinois) was used to predict harvest location based on the highest correlation value obtained for a given fish to a water station. For each predicted placement, the numerical difference between it and the actual known harvest station of the fish was determined (i.e., a fish placed by the Jaccard similarity matrix at station 8, while the actual harvest location was at station 6, would have a placement error of 2). These values were averaged to provide a mean station error for the physical placement of each fish. To test the significance of this value, fish profiles were randomly assigned to stations and individual fish were tested against the now-randomized fish profile to create a "random" assignment error rate; this randomization and assignment process was repeated 1000 times. A one-tailed *t* test was used to determine if the predicted harvest location of fish was more accurate than the randomized data sets (SPSS ver. 14.0, SPSS Inc.).

Fig. 1. Map showing the positions of the 10 sampling stations in the Irish and Celtic seas, November 2000. The number of fish harvested at each site is indicated in parentheses beside each station. The total distance between sampling stations is 717 km, with an average distance of 80 km (± standard error (SE) of 18) between adjacent stations. Scale bar indicates 100 km.



Using only shared water and outer-mucus TRFs, fish were placed within 3.00 (± standard error (SE) of 0.44) stations of their known harvest location, with 15.4% of fish placed to actual harvest location and 53.8% placed to the sea from which they were harvested (Table 1). The randomized data set based on shared outer-mucus and water TRFs placed fish within 3.05 (±SE 0.01) stations of their actual harvest location and 9.8% (±SE 0.15) were placed to their actual harvest location (Table 1). As such, there was no difference between the randomized station placement error and the real station placement error of fish ($p = 1.00$), although significantly more fish were placed to their exact harvest location than in the randomized data set ($p = 0.002$).

Using the shared water and mouth-mucus bacterial community, whiting were placed within 1.92 (±SE 0.31) stations of their known harvest location, with 27.8% of fish placed back to their exact harvest location, whereas 66.7% of fish were placed to the correct sea (Irish or Celtic) from which

Table 1. Average station placement error and the percentage of fish placed to correct harvest location based on real data and jack-knifed randomized data sets (significance (*p*) of placement evaluated using a one-tailed *t* test).

			Average station placement error			Fish placed to correct harvest location (%)		
Test fish	Comparison samples	*n*	Real[a]	Random[b]	*p*	Real	Random[c]	*p*
O	W	1000	3.00 (±0.44)	3.05 (±0.01)	1.00 (ns)	15.38	9.77 (±0.15)	0.002[d]
M	W	1000	1.92 (±0.31)	3.11 (±0.01)	0.002[d]	27.77	9.99 (±0.15)	0.002[d]
O	O	50	1.00 (±0.27)	2.90 (±0.05)	0.04[d]	61.54	7.68 (±0.69)	0.04[d]
M	M	50	1.55 (±0.28)	2.86 (±0.05)	0.04[d]	33.33	9.50 (±0.59)	0.04[d]
O + M	O + M	50	0.72 (±0.24)	2.87 (±0.05)	0.04[d]	69.44	8.86 (±0.77)	0.04[d]

Note: O, outer-mucus bacteria; M, mouth-mucus bacteria; W, water column bacteria; O + M, combined profiles; ns, not significant.

[a] Standard error (±) of the placement error for each fish from its capture location.

[b] Standard error (±) of the average placement error for all fish within each randomized data set.

[c] Standard error (±) associated with the average percentage of fish placed to correct harvest location within each randomized data set.

[d] Significant; lowest *p* value possible with a one-tailed significance test and the number of replicate randomizations performed.

the fish were harvested. The randomized data set for shared mouth mucus and water placed fish within 3.11 (±SE 0.01) stations of their known harvest location and placed only 10.0% (±SE 0.15) of fish to their known harvest location (Table 1). The placement of fish within 1.92 stations of their known harvest location is significantly more accurate for placing fish back to their harvest location than the randomized distribution (*p* = 0.002). The average distance between sampling sites was 79.7 km (±SE 17.6) (Fig. 1). In terms of actual distances, the placing of a fish within 1.92 stations of the known harvest location is equivalent to placing a fish to within approximately 153.0 km of its actual harvest location.

For the second test, total T-RFLP profiles from each fish were blindly compared with all other fish to predict harvest location. Three separate tests were carried out; T-RFLP profiles from (*i*) the outer mucus, (*ii*) the mouth mucus, and (*iii*) the combined outer mucus and mouth mucus of each fish were compared. The data were converted to presence or absence, and a Jaccard similarity matrix (SPSS ver. 14.0, SPSS Inc.) was used to predict harvest location. Individual fish were assigned to the harvest location of its highest scoring fish in the Jaccard similarity matrix. Mean station placement error was calculated as outlined above. The statistical significance of the placements was evaluated using a randomized placement test for each of the three tests. To perform these comparisons, aligned T-RFLP profiles from the fish outer mucus, mouth mucus, and combined outer and mouth mucus were converted to presence–absence data; the data were jack-knifed to ensure that individual fish were removed evenly from the analysis and then compared extrinsically using a Jaccard similarity matrix to place individual fish to one of the 10 harvest locations from which the mean station placement error was determined as described above. This was repeated 50 times for each of the three tests. A one-tailed *t* test was used to determine if the harvest location predictions were more accurate than the randomized data sets (SPSS ver. 14.0, SPSS Inc.).

Based on outer-mucus-only T-RFLP profiles, fish were placed, on average, within 1.00 (±SE 0.27) station of their actual harvest location, with 61.5% of the predicted fish placements to the actual harvest location. Station error was slightly higher when based on mouth-mucus-only T-RFLP profile comparisons, with fish placed within 1.55 (±SE 0.28) stations of their real harvest location and only 33.3% of fish placed back to their actual harvest location. The smallest station placement error was achieved using the combined outer-mucus and mouth-mucus T-RFLP profiles for each fish, resulting in a station placement error of 0.72 (±SE 0.24) from the known harvest location. Furthermore, the percentage of fish placed to their actual harvest location was greatest when both the outer- and mouth-mucus T-RFLP profiles for a single fish were used, resulting in 69.4% of fish placed to their actual fish harvest location and 88.9% of fish placed to the sea from which they were harvested. The fish placements were tested against randomized data sets (*n* = 50). All three placement tests fell statistically outside of the random distribution (*p* = 0.04) (Table 1); the *p* value stated is the highest significance value obtainable from a one-tailed *t* test carried out on a data set randomized 50 times. Using the average distance between stations, the placement of fish within 0.72 (±SE 0.24), 1.00 (±SE 0.27), and 1.55 (±SE 0.28) stations of their known harvest location is equivalent to identifying fish harvest location in the Irish and Celtic seas to within 57.3, 79.7, and 123.5 km, respectively.

The results of this study demonstrate the potential of the bacterial community associated with whiting to be used as biological tags to determine fish harvest location on a relatively small spatial scale. Our results have shown that the mucus layers of fish harvested from the same location have more TRFs in common than the mucus layers of fish harvested from other locations, supporting the previous observation by Wilson et al. (2008) of the geographical clustering of cod harvested from the Baltic, Icelandic, and North seas, also based on bacterial communities associated with the outer mucus layer. Whereas that study focused on large geographical distances, the current study was carried out in two neighbouring seas over a short distance (758 km between the 10 sampling stations), resolving fish placements within two stations, approximately 153 km, of their known harvest location based on shared water and mouth-mucus bacterial TRFs. The resolution of the method increased significantly when fish-to-fish comparisons of the bacterial community present in the mucus layers were used, with the highest resolution achieved using both the outer- and mouth-mucus bacterial community for each fish, placing fish, on average, to within a station of the known sampling station, equivalent to approximately 57 km. To our knowledge, no other method of predicting fish harvest location offers the spatial resolution demonstrated in this study.

Key advantages of exploiting bacteria as biological tags are the presence of diverse bacterial communities on marine fish (Smith et al. 2007; Wilson et al. 2008) that have been shown to vary spatially (this study; Wilson et al. 2008) and the high-throughput, culture-independent molecular methods used for their analysis. The sampling method is also favourable to field conditions. The only onboard processing is the snap-freezing of small, aseptically collected samples of the fish mucus layer. Subsequent laboratory analysis (DNA extraction, PCR amplification, and T-RFLP analysis) can be carried out in an automated high-throughput (Wilson et al. 2008) manner. In addition, the freely accessible software T-Align (Smith et al. 2005) allows for fast and accurate comparison of multiple T-RFLP profiles, further enhancing the accessibility of this method.

The results of this study highlight the potential use of the bacterial assemblages associated with marine fish as a reliable, fast, and relatively inexpensive biological tagging method that could easily be implemented to complement current techniques, such as parasitology, body morphology, and otolith structure – microchemistry, providing a powerful tool for fisheries management. As with other biological tags currently in use, the effectiveness and power of the method will be increased by combining complementary tools. Future work will assess the use of bacteria as biological tags in association with other established methods, over repeated sampling seasons and larger geographical areas. The technique could also be extended to include other fish species and commercially important stocks of crustaceans and shellfish.

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Microsatellites reveal clear genetic boundaries among Atlantic salmon (*Salmo salar*) populations from the Barents and White seas, northwest Russia

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Abstract: Fourteen microsatellite loci were employed to study the genetic structure of 34 Atlantic salmon (*Salmo salar*) populations from the White and Barents seas area, the last major European region where the species has remained in its natural state. The populations were separated into four distinct clusters (Atlantic Ocean and western Barents Sea, Kola Peninsula, western White Sea, and eastern Barents Sea) within which genetic divergence varied between 0.02 and 0.10 as estimated with F_{ST}. When this structuring was contrasted with previously identified mtDNA-based groupings, a remarkable similarity was observed, implying that these four groups can be considered as a good starting point for defining management units in the region. Indeed, several approaches for assessing every population's conservation value suggested that conservation of populations from each observed cluster would maximize preservation of the region's genetic diversity. Furthermore, each unit may require differing management strategies, as distinct patterns of genetic diversity and divergence characteristics were detected. In addition, individual assignment success within a region was high (87%–96%), indicating that the data can be used as a baseline to differentiate individuals caught in offshore fisheries on a regional level with a relatively high degree of accuracy.

Résumé : Quatorze locus microsatellites nous ont servi à étudier la structure génétique de 34 populations de saumons atlantiques (*Salmo salar*) des environs de la mer Blanche et de la mer de Barents, la dernière grande région d'Europe où l'espèce est demeurée dans son état naturel. Les populations se divisent en quatre regroupements distincts (Atlantique et mer de Barents occidentale, péninsule de Kola, mer Blanche occidentale et mer de Barents orientale) au sein desquels la divergence génétique, estimée par F_{ST}, varie de 0,02 à 0,10. Une comparaison de cette structure avec des groupements basés sur l'ADNmt identifiés précédemment montre une remarquable similarité, ce qui indique que ces quatre groupes forment un bon point de départ pour l'identification des unités de gestion dans la région. En effet, plusieurs méthodes utilisées pour évaluer la valeur de conservation de chaque population indiquent que la conservation de populations de chaque groupement observé permettrait de maximiser la conservation de la diversité génétique dans la région. De plus, il se peut que chaque unité nécessite des stratégies de gestion différentes puisqu'on détecte des patrons distincts de diversité génétique et des caractéristiques de divergence particulières. Enfin, le succès de l'attribution des individus à un groupement particulier est élevé (87 % – 96 %) dans une même région, ce qui indique que nos données peuvent servir de base pour l'identification des individus récoltés dans les pêches du large avec un degré relativement élevé d'exactitude à l'échelle régionale.

[Traduit par la Rédaction]

Introduction

Atlantic salmon (*Salmo salar*) is an economically highly valuable fish species that is threatened or has even gone extinct in many areas of its native distribution owing to numerous reasons such as building of power plant dams, pollution, badly designed stocking regimes, offshore overfishing, fish parasites such as *Gyrodactylus salaris*, clearing of rivers for timber floating, etc. (e.g., Parrish et al. 1998). The White and Barents seas in northwest Russia are among

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the last areas in Europe where Atlantic salmon populations are still stable (Parrish et al. 1998). The greatest threat to these populations is illegal fishing, although thus far, none of them have been severely affected by it. Hence, Atlantic salmon stocks of northwest Russia form an important source of biodiversity for the entire European region and are of immense economic and social importance for the local people in the form of fishing tourism. Currently, however, knowledge of the population genetic structure of Atlantic salmon populations from these regions is based primarily on a mtDNA study (Asplund et al. 2004) as well as several studies using nuclear markers, which included only a handful of populations from this region (e.g., Kazakov and Titov 1991; Makhrov et al. 2005; Tonteri et al. 2005). It would therefore be important to conduct a more detailed study of population genetic structure based on nuclear markers to provide information that could be used in designing management plans and to serve as a baseline to assign individuals caught in offshore fisheries to their populations of origin.

One factor that has had a big influence on the genetic structure of Atlantic salmon populations from the White and Barents Sea area is the glacial history of the region. During the last glaciation, a massive ice sheet covered the whole area for thousands of years until the ice receded approximately 11 500 – 12 000 years ago (e.g., Svendsen et al. 2004). The postglacial history of northwest Russian Atlantic salmon has been studied previously and different scenarios about recolonization have been suggested. Makhrov et al. (2005) proposed that the Barents Sea was colonized from both the eastern and western Atlantic Ocean and that the White Sea Basin was colonized by salmon from the Baltic Sea Basin in addition to immigrants from the Atlantic Ocean. A Baltic influence in the area was also suggested by Kazakov and Titov (1991). Asplund et al. (2004), however, suggested that the rivers of the White and Barents seas were colonized primarily from an eastern refugium with salmon from the Atlantic Ocean arriving to Kola Peninsula later on. The idea of an eastern refugium with additional immigrants from the west was further supported by Tonteri et al. (2005).

To date, studies of genetic population structure in Atlantic salmon have tended to focus at either a very broad or a very fine scale (King et al. 2007). For example, based on a variety of genetic markers, Atlantic salmon can clearly be divided into North American and European salmon (e.g., Ståhl 1987; Bermingham et al. 1991; King et al. 2001) and European Atlantic salmon further into two major groups, eastern Atlantic and Baltic salmon (e.g., Ståhl 1987; Bourke et al. 1997; Nilsson et al. 2001). Several studies investigating the population genetic structure of Atlantic salmon have included some populations from the White and Barents seas (e.g., Nilsson et al. 2001; Säisä et al. 2005; Tonteri et al. 2005) but too few to make detailed inferences about the population genetic structure between rivers within the particular region despite the fact that understanding population genetic structure at this scale is very important for management purposes.

Thus far, the only study with extensive sampling over the entire White and Barents Sea area was conducted by Asplund et al. (2004) who used mtDNA haplotype data from 28 populations in this region to identify clear changes in mtDNA haplotype lineage frequencies at several points along the White Sea and Kola Peninsula coasts, dividing the populations into three groups: western Barents Sea, Kola Peninsula, and White Sea. Although mtDNA has often been informative as a population genetic marker owing to its haploid and nonrecombining mode of inheritance and relatively rapid mutation rate, mtDNA is not without its limitations. Mitochondrial studies are commonly based on a small number of genes and always on just one locus, potentially leading to erroneous population genetic inference (e.g., Pamilo and Nei 1988). Furthermore, owing to the fourfold difference in effective population size between mtDNA and nuclear marker loci, the effect of random genetic drift is much stronger on mtDNA, which may be manifested as higher levels of divergence in mtDNA markers than in nuclear markers (Avise 2004). In addition, some empirical studies have shown that selection may complicate mtDNA patterns (Hey 1997). Therefore, to draw more robust population genetic conclusions, also studying the genetic relationships of the populations with nuclear markers is warranted.

Several studies have assessed allozyme variation in either White Sea or Kola Peninsula Atlantic salmon populations in more detail (Kazakov and Titov 1991; Makhrov et al. 2005). However, neither study covered the entire northwest Russian region nor identified any clear structuring among the analyzed populations, possibly owing to the lower level of variability observed at allozyme loci and the low number of polymorphic loci assessed (one to three polymorphic loci per study). Therefore, a detailed study of the genetic structuring of populations in the region with a moderate to large number of highly variable nuclear markers is still lacking.

Here, microsatellite analysis of an extensive set of Atlantic salmon populations from the White and Barents Sea area was carried out to gain insight into the population genetic structure based on nuclear markers that could be contrasted with the maternal genetic structure identified by Asplund et al. (2004). Additionally, we used the data set to assess the relative proportions of the region's overall genetic diversity, which can be used as a foundation for making management and conservation plans. Finally, the accuracy of the data as a reference for individual assignment in offshore fisheries was also examined.

Materials and methods

A total of 1352 Atlantic salmon individuals from 34 populations from the North Sea, Norwegian Sea, Barents Sea, and White Sea were analyzed (Fig. 1; Table 1). Population samples generally consisted of individuals collected from a single river stretch at one time point between 1996 and 2002 (with the vast majority being collected between 1999 and 2001) primarily by electrofishing and permanent traps. Most of the caught individuals were +0 to +4 fry or parr, rarely smolts or precocious males. Part of the samples from Tenojoki and all of the samples from Dee were adult salmon. River Kola samples consisted of hatchery-reared juveniles, while the rest were wild-caught.

Fin samples were stored in 95% ethanol and DNA was extracted using either the salt extraction method of Aljanabi and Martinez (1997) or the slightly modified silica-based protocol of Elphinstone et al. (2003). In the latter, 70 µL of digestion buffer (0.4 mol/L NaCl, 10 mmol/L Tris–HCl

Table 1. Details and microsatellite diversity of the studied Atlantic salmon populations.

Sampled populations									Microsatellite diversity			
					Stock size							
No.	Population	Abbreviation	River length (km)	Drainage area (km^2)	No. of ascending adults per year	Reference^a	Sampling year	N	A (range)	A_r	H_O	H_E
Northeast Atlantic Ocean and western Barents Sea												
1	Dee	Dee	126	2100	50000–100000	1	1996	32	12.4 (3–25)	8.4	0.74	0.78
2	Søya	Søy	25	152	100–500	2	2002	27	9.9 (4–22)	7.2	0.76	0.72
3	Reisa	Rei	130	2702	1000–5000	2	2002	14	7.4 (2–16)	6.8	0.66	0.68
4	Tenojoki	Ten	920	16386	50000–100000	3	1998	43	11.9 (3–28)	7.5	0.69	0.72
5	Titovka	Tit	79.1	1226.3	500–1000	4	2000	40	12.0 (2–30)	7.7	0.74	0.74
6	Zapadnaya Litsa	ZaL	98.9	1687.9	1000–5000	4	2000	40	9.7 (2–18)	6.8	0.75	0.73
7	Ura	Ura	73.4	1029.5	1000–5000	4	2000	41	11.2 (2–27)	7.4	0.71	0.72
8	Tuloma	Tul	59.8	21 216	5000–10000	4	2000	51	12.1 (3–30)	7.6	0.73	0.76
9	Kola	Kola	83	3845.6	5000–10000	5	2000	42	12.1 (4–28)	7.7	0.76	0.75
10	Drozdovka	Dro	53.4	258	100–500	4	2001	40	8.9 (2–19)	6.3	0.75	0.70
11	Yokanga	Yok	202.7	5944	5000–10000	6	2001	40	11.4 (3–25)	7.4	0.73	0.73
Eastern and southern Kola Peninsula												
12	Kachkovka	Kac	59.4	842.6	1000–5000	4	2001	41	11.9 (4–30)	7.5	0.70	0.73
13	Ponoi Lebyazhya^b	Leb	57.2	714.3	10000–50000/500–1000	7	2001	47	12.1 (3–31)	7.3	0.73	0.72
14	Ponoi Pacha^b	Pac	22.2	156.3	10000–50000/<100	7	2001	38	10.1 (3–27)	6.8	0.73	0.72
15	Danilovka	Dan	38.8	262.2	100–500	4	2001	44	10.9 (2–29)	7.1	0.72	0.71
16	Babya	Bab	41.5	348.3	500–1000	4	2001	46	12.8 (2–36)	7.7	0.71	0.73
17	Lihodeevka	Lih	37	307.8	100–500	4	2001	47	11.7 (2–30)	7.3	0.71	0.71
18	Pulonga (Kola Peninsula)	PuK	77.5	730	500–1000	8	2001	47	11.8 (4–28)	7.2	0.70	0.71
19	Yugin	Yug	43.8	206.8	<100	4	2001	56	11.2 (3–28)	7.0	0.70	0.71
20	Kitsa	Kit	51.4	1650	1000–5000	9	2000	37	11.2 (3–34)	7.2	0.76	0.72
21	Varzuga Yaprema^b	Yap	28.2	179.9	10000–50000/<100	9	2001	40	12.1 (3–34)	7.4	0.72	0.74
22	Olenitsa	Ole	62.6	402.8	500–1000	4	2000	34	7.4 (1–19)	5.4	0.65	0.63
23	Umba	Umb	124.8	6248.5	5000–10000	10	2001	47	11.1 (3–30)	6.9	0.72	0.71
24	Pila	Pil	47.1	322.9	100–500	4	2000	35	7.5 (2–16)	5.4	0.68	0.65
25	Kolvitsa	Kolv	8.7	1310	100–500	4	2000	41	9.1 (2–21)	6.5	0.74	0.69
Western White Sea												
26	Nilma	Nil	16.1	164.5	<100	7	1999	42	5.3 (2–9)	4.4	0.71	0.65
27	Pulonga (White Sea)	PuW	52	640.9	100–500	7	1999	43	5.6 (1–11)	4.5	0.63	0.61
28	Pongoma	Pon	103.1	1200	100–500	7	1999	50	8.4 (2–18)	6.0	0.67	0.68
29	Suma	Sum	157.5	2041	500–1000	7	2001	41	5.9 (2–13)	4.5	0.63	0.60
Eastern White and Barents seas												
30	Severnaja Dvina Emtsa^b	Emt	188	14 100	10000–50000/500–1000	11	2001	42	9.6 (3–19)	6.6	0.72	0.71
31	Severnaja Dvina Padoma^b	Pad	68	499	10000–50000/100–500	11	2001	48	6.5 (2–13)	4.8	0.62	0.61
32	Megra	Meg	119	2180	500–1000	11	2001	43	10.1 (3–24)	6.8	0.69	0.70
33	Pechora Pizhma^b	Piz	380	5470	10000–50000/1000–5000	12	1999	22	6.7 (3–14)	5.5	0.68	0.67
34	Pechora Unja^b	Unj	163	2890	10000–50000/500–1000	12	1999	11	5.2 (2–14)	5.2	0.63	0.62

Note: No., population number; N, number of individuals; A, average number of alleles in a population; A_r, allelic richness; H_o, observed heterozygosity; H_e, expected heterozygosity.

[a] 1, J. Taggart, Institute of Aquaculture, University of Stirling, Stirling FK9 4LA,UK, personal communication; 2, Statistics Norway (www.ssb.no); V. Wennevik, Institute of Marine Research, P.O. Box 1870 Nordnes, N-5817 Bergen, Norway, personal communication; 3, J. Erkinaro, Finnish Game and Fisheries Research Institute, Tutkijantie 2A, FIN-90570 Oulu, Finland, personal communication; 4, Zubchenko et al. (2007*a*); 5, Zubchenko et al. (2003); 6, Dolotov (2007); 7, A.Je. Veselov, unpublished data; 8, Kaluzchin (2003); 9, Zubchenko et al. (2002); 10, Zubchenko et al. (2007*b*); 11, Studenov (1997); 12, Martynov (2007).

[b] Samples were collected from a single tributary of a larger river system; therefore, two stock size estimates are given: one for the entire river system and another for the specific tributary.

Table 2. Locus references, PCR details, and genetic diversity indices of the microsatellite markers employed in the study.

		PCR details			Diversity indices						
Locus	Microsatellite reference	Primer concentration (pmol/L)	Primer sequences	Label	Size range (bp)	Total *A*	Mean *A*	A_r (11)	H_o	H_e	F_{ST}
Qiagen multiplex I											
Ssa412UOS	Cairney et al. 2000	0.2	F: GTGGAGATACACAGCACTTA R: GTTTCTTGGTTAGTACCGGACATG*	FAM	282–298	9	3.7	3.4	0.56	0.57	0.12
CA063557	Vasemägi et al. 2005	0.04	F: CACAGGCACACACTCCTCAT R: GTTTCAGGTGAAGAGCATGACCAA	FAM	341–373	13	2.9	2.5	0.27	0.26	0.07
Ssa197	O'Reilly et al. 1996	0.04	F: TGGCAGGGATTTGACATAAC R: GTTTGGCAGGAGTCAGGTG*	VIC	217–329	27	13.3	11.4	0.86	0.88	0.05
Ssa405UOS	Cairney et al. 2000	0.3	F: CTGAGTGGGAATGGACCAGACA R: GTTTACTCGGGAGGCCCAGACTTGAT	VIC	288–404	29	17.7	13.4	0.91	0.90	0.05
Ssa98	P. O'Reilly et al., unpublished†; GenBank accession No. AF019195	0.2	F: GTTTGCTGTTGTCATTTGCAGTCC‡ R: GGCATCTGTAGTTGGGCAAG‡	NED	435–479	15	5.6	4.6	0.45	0.45	0.04
CA059983	Vasemägi et al. 2005	0.025	F: GCGGCCCTTAGTGTAATCAA R: GTTTCTCGCCAGTCACTCTTCAA	PET	265–285	11	4.9	4.6	0.61	0.60	0.09
CA060177	Vasemägi et al. 2005	0.25	F: CGCTTCCTGGACAAAAATTA R: GTTTCAAACCCCTGTCATGTTTCA*	PET	386–482	38	15.6	12.5	0.89	0.88	0.06
Qiagen multiplex II											
SSOSL311	Slettan et al. 1995	0.4	F: TAGATAATGGAGGAACTGCATTCT R: GTTTCATGCTTCATAAGAAAAAGATTGT	FAM	106–184	33	13.0	10.5	0.82	0.81	0.08
Ssa171	O'Reilly et al. 1996	0.2	F: ATTATCCAAAGGGGTCAAAA R: GTTTGAGGTCGCTGGGGTTTACTAT	FAM	188–256	25	9.7	7.9	0.79	0.76	0.06
Ssa85	O'Reilly et al. 1996	0.015	F: GTTTAGGTGGGTCCTCCAAGCTAC R: ACCCGCTCCTCACTTAATC	VIC	113–167	23	9.6	8.5	0.79	0.79	0.06
Ssa404UOS	Cairney et al. 2000	0.07	F: GTTTGAAGTTCAGACAGCAAAGCA* R: CTGCATATCTCTCCCTCTCCA*	VIC	379–511	70	23.3	16.2	0.92	0.93	0.04
Ssa202§	O'Reilly et al. 1996	0.6	F: GTTTCTTGGAATATCTAGAATATGGC R: TTCATGTGTTAATGTTGCGTG	NED							
SsaA124	King et al. 2005	0.025	F: TGCACCTGACTTCTATTCCAGTA* R: GTTTAACTGGGCCACAGGCTATC*	PET	180–228	12	3.7	3.6	0.56	0.56	0.07
Normal multiplex											
SSOSL438	Slettan et al. 1996	0.1	F: GACAACACACAACCAAGGCAC R: GTTTATGCTAGGTCTTTATGCATTGT	NED	111–149	17	5.7	5.1	0.67	0.66	0.09
SSOSL85	Slettan et al. 1995	0.25	F: TGTGGATTTTTGTATTATGTTA R: GTTTCCTCATTCAGTACCTTCACC*	NED	163–207	18	8.5	7.8	0.77	0.74	0.08

Note: Total *A*, total number of alleles observed across populations; mean *A*, average number of alleles per population; A_r (11), allelic richness; H_o, average observed heterozygosity within a population; H_e, average expected heterozygosity within a population.

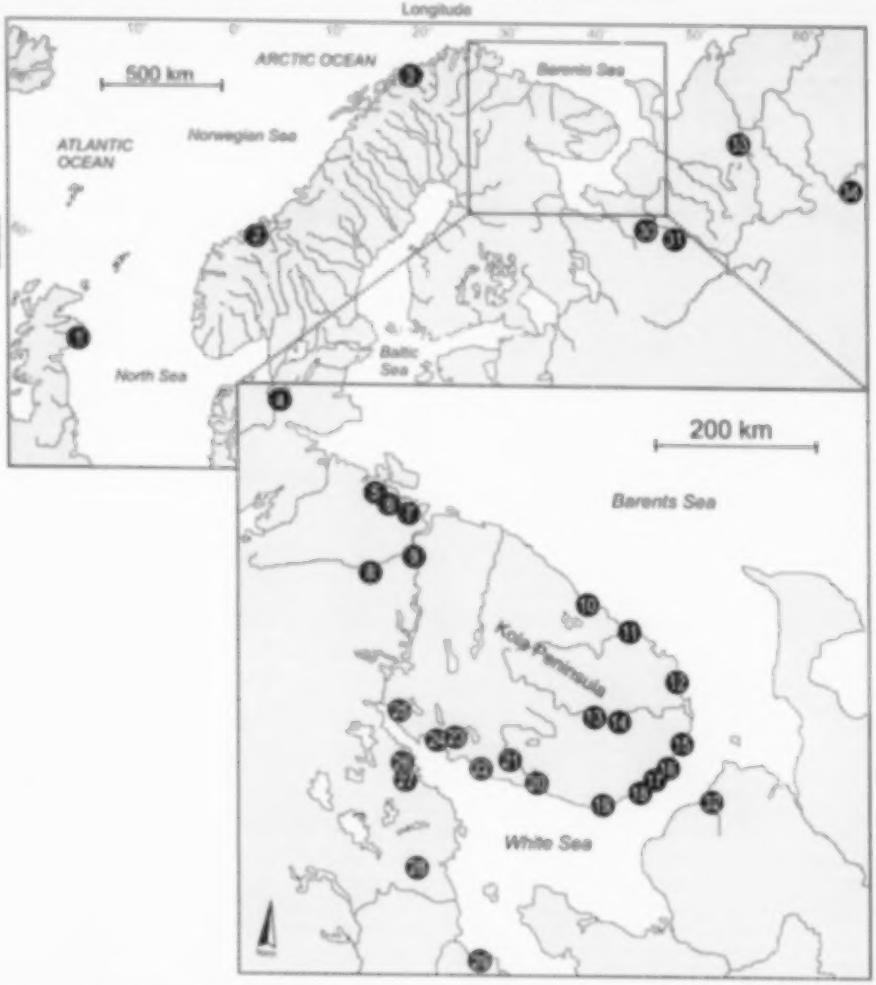
*A new primer was designed to obtain a shorter or longer PCR product than with the published primers.

†Patrick O'Reilly et al., Fisheries and Oceans Canada, Bedford Institute of Oceanography, One Challenger Drive, Dartmouth, NS B2Y 4A2, Canada, unpublished data.

‡No published primer sequences were available; thus, novel primers were designed for the locus.

§The locus was included in the PCR but was later excluded from all analyses owing to difficulties in genotyping.

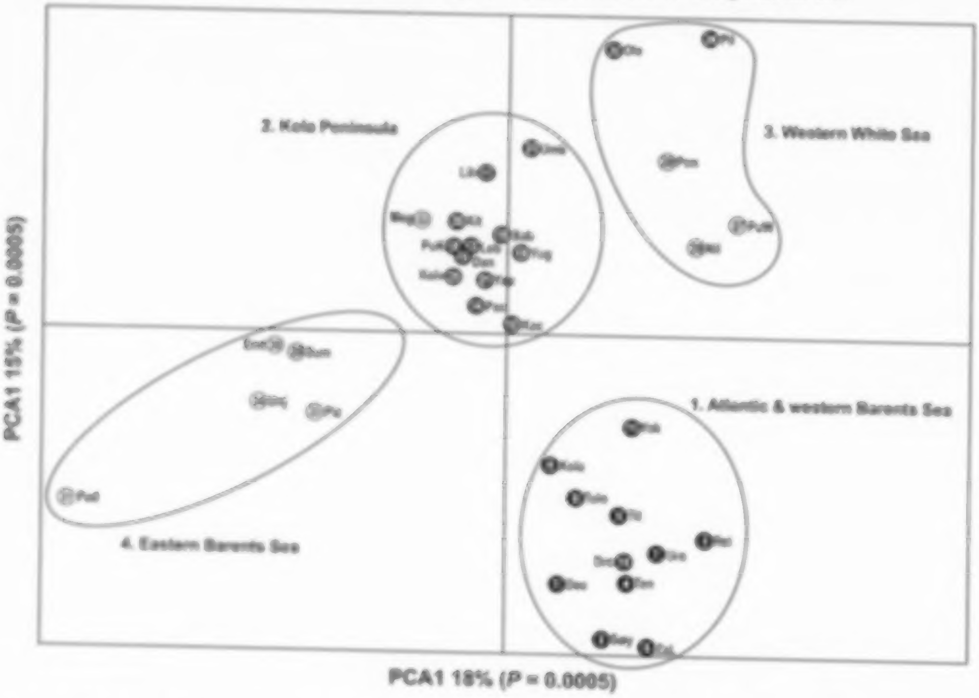
Fig. 1. Map of the sampling locations of the studied populations. See Table 1 for population names.



(pH 8.0), 2 mmol/L EDTA (pH 8.0), and 2% SDS) containing 28 µg of proteinase K (Finnzymes) was pipetted into the wells of a 96-well PCR plate after which a 2 mm^3 piece of tissue was added into each well and the plate incubated at 55 °C for 3 h. Following the digestion, the protocol continued as described in Elphinstone et al. (2003).

Initially, 15 microsatellite loci were screened in the study (see Table 2 for locus details). For six of the loci, either one or both of the primers were redesigned to obtain a longer or shorter PCR product than what would have been obtained with the published primers. To facilitate genotyping by means of enhancing 3′ adenylation, a GTTT "PIGtail" (Brownstein et al. 1996) was added to the 5′ end of each nonlabeled primer. The Qiagen multiplex PCR kit was utilized in the amplification of 13 loci in two 10 µL reactions consisting of approximately 50 ng of extracted DNA, 0.015–0.6 µmol/L each primer, one of which was fluorescently labeled (Table 2), and 1× Qiagen multiplex PCR master mix (Table 2). The Qiagen multiplex PCR protocol for subset I of these loci (*Ssa412UOS*, *CA063557*, *Ss197*, *Ssa405UOS*, *Ssa98*, *CA039983*, and *CA060177*) started with a 15 min initial activation step at 95 °C followed by 35 cycles of denaturation at 94 °C for 30 s (s), annealing at 62 °C for 90 s, and extension at 72 °C for 60 s and a final extension at 60 °C for 30 min. For subset II of the loci (*SSOSL311*, *Ssa171*, *Ssa85*, *Ssa404UOS*, *Ssa202*, and *SsaA124*), the protocol was essentially the same except that for the first 11 cycles, the annealing temperature was 61 °C and for the following 25 cycles was 60 °C. The two remaining loci (*SSOSL85* and *SSOSL438*) were amplified in a 10 µL reaction consisting of approximately 50 ng of DNA, 0.25 and 0.1 µmol/L each primer (*SSOSL85* and *SSOSL438*, respectively), one of which was fluorescently labeled (Table 2), 1× NH_4 reaction buffer (160 mmol/L $(NH_4)_2SO_4$, 670 mmol/L Tris–HCl (pH 8.8), and 0.1% Tween-20) (Bioline), 1.5 mmol/L $MgCl_2$, 250 µmol/L dNTP, and 0.1 U of BioTaq DNA polymerase (Bioline). The touchdown PCR protocol for these two loci started with a 3 min denaturation at 94 °C ensued by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55–45 °C (0.5 °C decrease per cycle until

Fig. 2. Result of the PCA. Circle colors indicate the geographical origin of each population as follows: solid circles, populations from the northeast Atlantic Ocean and western Barents Sea; dark-shaded circles, populations form the eastern and southern Kola Peninsula; light-shaded circles, populations from the western White Sea; open circles, populations from the eastern White and Barents seas (see Table 1 and Fig. 1 for details). The four clusters of populations represent the genetic clusters referred to throughout the text.



45 °C was reached) for 30 s, and extension at 72 °C for 30 s and a final extension at 72 °C for 5 min. All of the loci have also been amplified in single-locus PCRs on a subset of the individuals to verify the genotypes. All amplifications were performed on PTC100, PTC200 (MJ Research), or Mastercycler gradient (Eppendorf) thermal cyclers.

For electrophoresis, 2 µL of the Qiagen subset I PCR product, 4 µL of the Qiagen subset II PCR product, and 4 µL of the *SSOSL85–SSOSL438* PCR product were pooled together and diluted with 70 µL of sterile water. An aliquot of 2 µL was combined with 0.1 µL of GS600LIZ size standard (Applied Biosystems) and 9.95 µL of HiDi-formamide. The samples were denaturated for 3 min at 95 °C and then electrophoresed on an ABI Prism 3130*xl* genetic analysis instrument. GeneMapper 4.0 (Applied Biosystems) was used to analyze the DNA fragments and to score the genotypes. All genotypes were inspected visually after which data were exported to a spreadsheet program for further analysis excluding, however, locus *Ssa202* owing to difficulties in genotyping. In total, 1352 individuals were successfully genotyped witn at least 11 of the remaining 14 microsatellite loci.

GENEPOP version 3.4 (Raymond and Rousset 1995) was utilized to test for significant deviations from the Hardy–Weinberg equilibrium or genotypic linkage equilibrium and to correct for multiple significance tests; a sequential Bonferroni type method ($\alpha' = 1 - (1 - \alpha)^{1/k}$) (Rice 1989; Gotelli and Ellison 2004) was employed. The potential presence of genotyping errors such as null alleles and large allele dropout was examined with MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004). The observed number of alleles (A) and the observed and expected heterozygosities (H_o and H_e, respectively) were estimated using Microsatellite Toolkit 3.1 (Park 2002) and GENECLASS2 (Piry et al. 2004), and in addition, to account for unequal sample sizes, allelic richness (A_r) based on a minimum sample size of 11 diploid individuals was calculated with FSTAT 2.9.3.2 (Goudet 2001). The latter program was also employed to study genetic divergence among the populations by calculating the θ estimator (Weir and Cockerham 1984) of Wright's F_{ST} over all populations and for all population pairs. Statistical significance of pairwise F_{ST} estimates was assessed with the pairwise test of differentiation implemented in FSTAT 2.9.3.2, which also performs a strict, not sequential, Bonferroni correction.

To examine if any of the populations have recently experienced reductions in their effective population size (N_e), the Wilcoxon's test, which is appropriate when <20 loci are used (Piry et al. 1999), and the graphical mode shift indicator as implemented in BOTTLENECK 1.2.02 (Piry et al. 1999) were employed. As recommended by Piry et al. (1999), the loci were assumed to evolve under the two-phase mutation model (Di Rienzo et al. 1994) with 5% of the mutations involving multiple steps with a variance of 12. Statistical significance of the Wilcoxon's test was achieved by 2000 replications and the sequential Bonferroni correction for multiple significance tests was carried out as described above.

Fig. 3. Relationship between the geographical distance and genetic distance ($F_{ST}/(1 - F_{ST})$) of the studied Atlantic salmon populations. Squares represent interpopulation distances between populations among each PCA group (solid, Atlantic Ocean and western Barents Sea; dark shaded, Kola Peninsula; light shaded, western White Sea; open, eastern Barents Sea) and crosses represent interpopulation distances between the four groups. The line depicts the regression slope of all interpopulation comparisons (Mantel's $r_{XY} = 0.39$, $P = 0.008$).

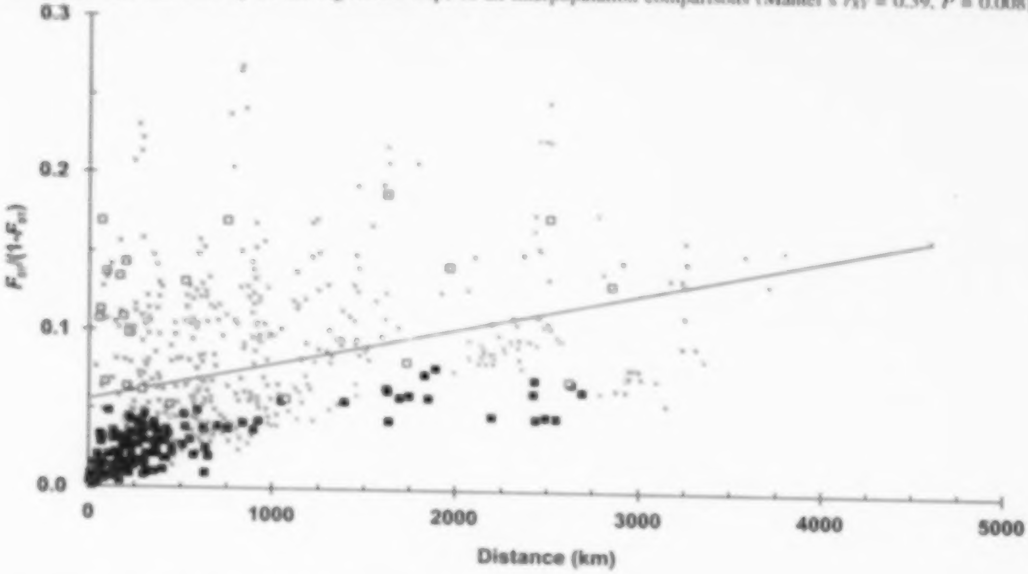
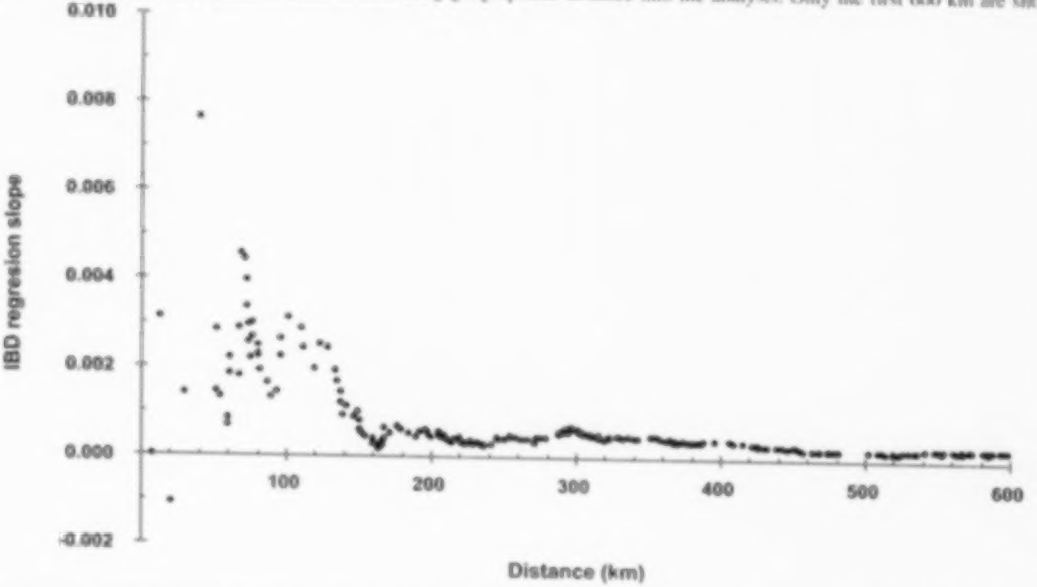


Fig. 4. Relationship between the slope of isolation by distance (IBD) regression and the geographic distance between the populations. Each slope was calculated by adding population pairs at increasing geographical distance into the analysis. Only the first 600 km are shown.



To study interpopulation relationships, a principal components analysis (PCA) was performed using PCA-GEN 1.2 for Windows developed by Jérôme Goudet (available at www2.unil.ch/popgen/softwares/pcagen.htm) with statistical significance of the axes being obtained by 2000 randomizations. The group permutation test implemented in FSTAT 2.9.3.2. was utilized to compare genetic diversity (A_r, H_o, and H_e) and genetic divergence (F_{ST}) among the four clusters defined by the PCA (Fig. 2). Statistical significance of the comparisons was obtained by 2000 permutations. In addition, Arlequin 2.000 (Schneider et al. 2000) was employed to perform a hierarchical analysis of molecular variance (AMOVA) on four groups formed of the populations either based on their geographical distribution (Table 1) or according to the results of the PCA (Fig. 2).

Fig. 5. Proportion of genetic diversity (GD %) maintained in various groupings of the studied populations as estimated with Conserve 1.4b. For each PCA group, the pair retaining the greatest share of genetic diversity and the increase in diversity with successive additions of populations are indicated. For example, in the Atlantic Ocean and western Barents Sea group, Drozdovka, Zapadnaya Litsa, Yokanga, and Tuloma together retain 17.6% of the total genetic diversity, while adding Titovka to the group increases GD % to 19.9%. The last bar depicts the GD % preserved in the pairs with the highest GD % from each group together.

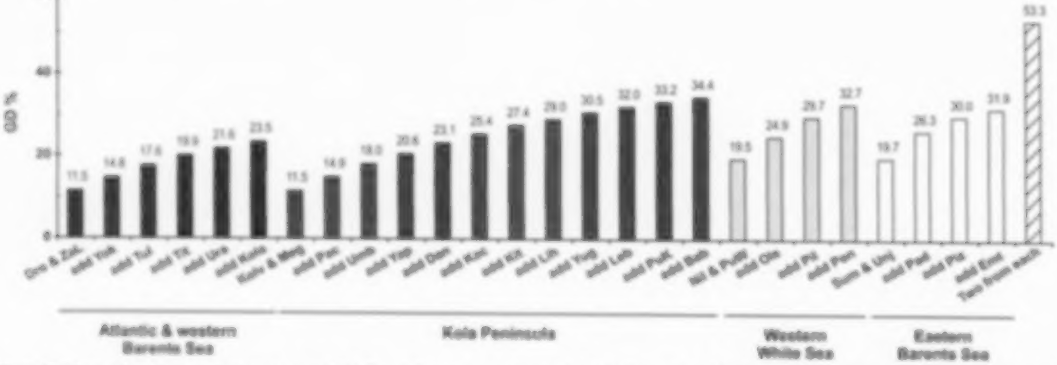
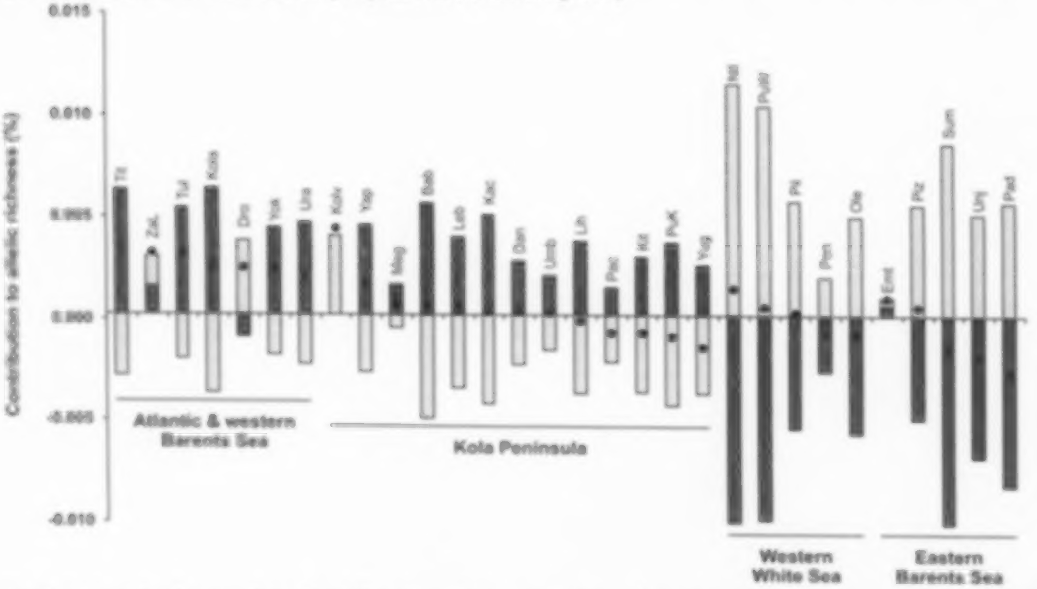


Fig. 6. Contribution of each population to total allelic richness as estimated with Contrib 1.02. The total contribution (C_T^r) of a population is denoted with a circle and the two components of variation, genetic diversity and population divergence, with bars (dark-shaded bar, C_S^r; lightshaded bar, C_D^r). Populations within a PCA group are ordered according to C_T^r.



A Mantel's test as implemented in GenAlEx 6 (Peakall and Smouse 2006) was utilized to examine whether an association between the geographical distance and genetic divergence of the populations existed (i.e., isolation by distance (IBD)). Interpopulation geographical distances were calculated as the shortest waterway distances between sampling locations and pairwise genetic divergence was estimated as $F_{ST}/(1 - F_{ST})$. The analysis was performed on all of the populations and separately for the four groups obtained from the PCA (Fig. 2) with statistical significance being assessed with 9999 random permutations. To study if the strength of IBD was constant over different geographical ranges, the slope of the IBD regression was calculated by including population pairs of increasing geographical distance one-by-one into the analysis. For example, all of the populations separated by 200 km or less were included in the calculation of the 200 km regression slope, and by adding the next pair to the analysis, a slope at 205 km was obtained.

The efficiency with which individuals could be assigned to their population of origin based on their multilocus genotype was inferred using the self-assignment method implemented in GENECLASS2 (Piry et al. 2004). The direct assignment approach was employed, and for likelihood estimation, the Bayesian method of Rannala and Mountain (1997) was chosen.

To assess the conservation value of the Russian Atlantic salmon populations included in this study, Conserve 1.4b (Crozier et al. 1999) was employed to estimate the percentage of total genetic diversity (GD %) retained in various groups formed of the populations. A treefile containing 100 neighbour-joining trees based on Cavalli-Sforza and Edwards' (1967) chord distance was created using the programs Seqboot, Gendist, and Neighbour from the PHYLIP 3.66 software package (Felsenstein 2006) and used as input for Conserve 1.4b. The following calculations were performed separately for each cluster observed in the PCA (Fig. 2). First, all possible combinations of two populations were formed and the genetic diversity retained in the pairs was calculated. Then, all possible combinations of three populations were formed by adding a third population to the pair preserving most variation, and again, GD % was calculated, after which all possible combinations of four populations were formed as explained above. This successive addition of populations to the group containing the greatest share of diversity was repeated until a group containing all of the populations was formed, and thus, the fraction of genetic diversity maintained in all of the populations forming one PCA cluster was obtained.

In addition, Contrib 1.02 (Petit et al. 1998) was utilized to estimate the contribution of each population to the total allelic richness among all of the Russian populations (C_T^r) and to partition this contribution into two components: a population's contribution to total allelic richness owing to (*i*) its own diversity (C_S^r) and (*ii*) its divergence from the other populations (C_D^r). As recommended by Petit et al. (1998), measures based on allelic richness were chosen over measures related to gene diversity, as allelic richness is highly dependent on effective population size and should thus reflect past demographic changes well.

Results

The average number of alleles per locus within a population ranged from 5.2 (Pechora Unja) to 12.8 (Babya), while allelic richness varied between 4.4 (Nilma) and 8.4 (Dee) (Table 1). The observed heterozygosity ranged from 0.62 (Severnaja Dvina Padoma) to 0.76 (Søya, Kola, and Kitsa) and the expected heterozygosity from 0.60 (Suma) to 0.78 (Dee) (Table 1). Over all loci, a total of 340 alleles were observed, with *Ssa404UOS* being the most variable with 70 alleles and *Ssa412UOS* the least variable with nine alleles (Table 2). *Ssa404UOS* is a locus with a tetranucleotide repeat motif but a high incidence of alleles that differ from one another by just 1 bp, hence explaining the high allele number. Despite this, the alleles were generally easily distinguishable from one another.

Four loci (*CA060177*, *Ssa404UOS*, *Ssa197*, and *SSOSL85*) were found to differ from Hardy-Weinberg proportions after sequential Bonferroni correction but were not excluded from further analysis because no consistent pattern of disequilibrium was observed as in all four loci and the deviation was due to both heterozygote deficiency and excess and mostly in different populations (supplementary Appendix S1).[3] At the population level, the Zapadnaya Litsa, Tuloma, and Pulonga Kola populations were found to be in Hardy–Weinberg disequilibrium after correcting for multiple significance tests, but again, these were regarded as negligible, as they affected just a few loci and both heterozygote deficiency and excess were observed. Across all populations, 29 locus pairs out of 91 pairs in total were found to be in significant linkage disequilibrium after correcting for multiple significance tests. As these disequilibria were observed only in eight populations or less, it is unlikely that they affected our analyses. However, a few populations were observed to have more than 10 of these 29 locus pairs in linkage disequilibrium: Zapadnaya Litsa 15, Pulonga White Sea 13, Kolvitsa and Ponoi Pacha 11, and Nilma 10. It therefore seems likely that the observed linkage disequilibrium is due to population-specific, rather than locus-specific, effects.

None of the loci displayed patterns of variability consistent with large allele dropout. However, significant excess of homozygotes implying the possible presence of null alleles was identified in seven loci. In most cases (*CA039983*, *Ssa404*, *Ssa412*, *SSOSL85*, and *SSOSL438*), the excess was observed in only one population; two loci (*CA060177* and *Ssa197*) had an excess of homozygotes in two different populations. River Teno was the only population that potentially had null alleles in two different loci. As the estimated frequency of null alleles was low in all cases (0.03–0.14) and as none of the loci displayed significant excess of homozygotes in more than two populations (out of 34), it is most likely that these observations do not represent true null alleles but are rather due to other factors.

After correcting for multiple significance tests, the Wilcoxon test did not reveal any populations that might have gone through recent population bottlenecks. Likewise, no mode shift in allele frequencies was observed, as in each population, the number of alleles in the low-frequency class (<0.1) was higher than the number of alleles in higher frequency classes, i.e., the frequency distributions were L-shaped (Luikart et al. 1998).

The PCA divided the populations into four clusters corresponding reasonably well to the geographical sampling regions (Fig. 2). The populations from the northeast Atlantic Ocean and western Barents Sea formed a clearly separate group. Likewise, all of the populations from eastern and southern Kola Peninsula grouped tightly together except for the populations Olenitsa and Pila, which clustered together with the populations from the western coast of the White Sea forming the third group. The fourth group was formed by populations from eastern White and Barents seas with the exception of the River Megra population, which grouped together with the geographically closer populations from the eastern Kola Peninsula. The River Suma population from the southern White Sea grouped together with the populations from the eastern White and Barents seas. These groups are subsequently referred to as the Atlantic Ocean and western Barents Sea group, the Kola Peninsula group, the western White Sea group, and the eastern Barents Sea group, respectively.

No difference in genetic diversity was observed between the Atlantic Ocean and western Barents Sea group (A_r = 7.3, H_o = 0.73, H_e = 0.73) and the Kola Peninsula group (A_r

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[3] Supplementary data for this article are available on the journal Web site (cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3896. For more information on obtaining material, refer to cisti-icist.nrc-cnrc.gc.ca/eng/ibp/cisti/collection/unpublished-data.html.

= 7.1, H_o = 0.71, H_e = 0.71) or between the western White Sea group (A_r = 5.1, H_o = 0.67, H_e = 0.65) and the eastern Barents Sea group (A_r = 5.3, H_o = 0.66, H_e = 0.64). However, the western White Sea group was significantly less variable than the Atlantic Ocean and western Barents Sea group (all $P < 0.003$) and the Kola Peninsula group (all $P < 0.02$). Likewise, the eastern Barents Sea group showed less genetic diversity than the Atlantic Ocean and western Barents Sea group (all $P < 0.002$) or the Kola Peninsula group (all $P < 0.006$).

Single-locus estimates of F_{ST} ranged between 0.04 (*Ssa98* and *Ssa40UOS4*) and 0.12 (*Ssa412UOS*) (Table 2) with the global estimate over all loci being 0.07. The lowest pairwise estimate of genetic differentiation was 0.001 (Babya-Pulonga Kola Peninsula) and the highest 0.211 (Severnaja Dvina Padoma-Pulonga White Sea) (Appendix A). The average pairwise F_{ST} within each of the four groups was as follows: Atlantic Ocean and western Barents Sea, F_{ST} = 0.032; Kola Peninsula, F_{ST} = 0.022; western White Sea, F_{ST} = 0.101; eastern Barents Sea, F_{ST} = 0.108. These within-group levels of genetic differentiation were significantly different between either the western White Sea group or the eastern Barents Sea group compared with either of the other two groups (P = 0.023–0.005), but the F_{ST} levels of the western White and eastern Barents Sea groups were not significantly different from each other, nor were the differences between the other two groups ($P \geq 0.66$). In the hierarchical AMOVA, the proportion of variation residing among groups was 20% higher when the populations were grouped according to the result of the PCA (3.1%, $P \leq 0.001$) rather than based on their geographical location (2.6%, $P \leq 0.001$) (Table 3). Most of the variation, approximately 93% ($P \leq 0.001$), resided at the within-population level.

The Mantel test revealed a significant association between the geographical and genetic distance between the populations (Mantel's r_{XY} = 0.39, P = 0.008) (Fig. 3). However, when it was examined if there was IBD within each of the groups revealed by the PCA (Fig. 2), a significant association was found only among the Atlantic Ocean and western Barents Sea and Kola Peninsula groups (Mantel's r_{XY} = 0.81 and 0.56, respectively, both P = 0.002), while the associations among the populations forming the western White and eastern Barents Sea groups were nonsignificant (Mantel's r_{XY} = –0.23 and 0.18, respectively, both P = 0.3). When IBD was examined on various geographical scales, a great deal of variation in the slope of the regression was found if only populations separated by less than 100 km were considered (Fig. 4). When population pairs separated by more than 100 km were included in the analysis, the IBD regression slope decreased as a function of increasing geographical distance until approximately 160 km, after which the slope plateaued out (Fig. 4).

Population-level self-assignment success varied considerably between populations. Considering populations from the Atlantic Ocean and western Barents Sea and Kola Peninsula groups, the success varied between 15.2% (Babya) and 87.8% (Kolvitsa), averaging 53%, while among populations from the western White and eastern Barents Sea groups, the assignment efficiency was generally higher, varying from 72.7% (Unja) to 100% (Nilma and Pulonga White Sea) and averaging 93% (Table 4). At the group level, assignment success was relatively high in all groups (87.3%–95.6%) (Table 4), indicating that the majority of misassignments at the population level were due to individuals being assigned to other populations from the same group.

Considering the share of diversity contained in different groups of Russian Atlantic salmon as calculated with Conserve 1.4b, populations from the Kola Peninsula group retained the highest GD %, 34.4% (Fig. 5). Within the group, populations from Kolvitsa and Megra formed the most diverse pair (GD % = 11.5%), and adding Ponoi Pacha to the pair raised the percentage the most to 14.9% (Fig. 5). Populations from the western White Sea group retained 32.7% and populations from and the eastern Barents Sea group 31.9% of the overall genetic diversity (Fig. 5). Within the western White Sea group, populations from Nilma and Pulonga White Sea maintained the highest GD % (19.5%), which increased to 24.9% after the addition of the Olenitsa population (Fig. 5). The Suma and Pechora Unja populations from East Barents Sea group formed a pair that retained 19.7% of the genetic diversity and adding Severnaja Dvina Padoma to the pair raised the percentage to 26.3% (Fig. 5). The GD % maintained among the Russian Atlantic salmon populations from the western Barents Sea group was approximately 30% lower than among the other groups (GD % = 23.5%) (Fig. 5). Within the group, Drozdovka and Zapadnaya Litsa retained 11.5% of the genetic diversity, which was raised to 14.8% by adding Yokanga (Fig. 5). Overall, the pairs maintaining the greatest share of diversity from each PCA group together retained 53.3% of the total genetic diversity (Fig. 5).

The populations contributing most to total allelic richness were partly the same as the populations retaining the most genetic diversity as estimated from the above Conserve 1.4b analysis: considering the three populations identified to retain the most genetic diversity within each of the four PCA groups (i.e., 12 populations in total), 6 out of these 12 populations were the same with both analysis methods (Figs. 5 and 6). Considering the Contrib 1.02 analysis, which takes the contributions of both genetic diversity as well as population divergence into consideration, there was a clear contrast between the two western/northern groups (the Atlantic Ocean and western Barents Sea group and the Kola Peninsula group) and the two southern/eastern groups (the western White Sea group and the eastern Barents Sea group). Apart from a few exceptions, genetic diversity was the main component contributing to allelic richness among the Atlantic Ocean and western Barents Sea and Kola Peninsula groups, while among the western White and eastern Barents Sea groups, genetic divergence was more influential (Fig. 6).

Discussion

Based on microsatellite variation, Atlantic salmon populations from the western Barents Sea clustered together with the northeastern Atlantic populations into a distinct cluster, while populations from the White and eastern Barents seas were separated into three additional groups. The clusters found here correspond very well to the geographical sampling regions and are well in line with the three mtDNA-based groupings described in Asplund et al. (2004). The

Table 3. Results of the hierarchical AMOVA for four geographical regions, northeast Atlantic Ocean and western Barents Sea, eastern and southern Kola Peninsula, western White Sea, and eastern White and Barents seas (Table 1), and the four groups revealed by PCA (see Fig. 2).

	Among groups			Within groups			Within populations		
Partitioning of the populations	%	*P*	F_{CT}	%	*P*	F_{SC}	%	*P*	F_{ST}
Geographical	2.6	***	0.03	4.6	***	0.05	92.8	***	0.07
Based on the PCA	3.1	***	0.03	4.1	***	0.04	92.7	***	0.07

Note: Percentages of total variation explained (%) and fixation indices are given for three hierarchical levels. $^{***}P \leq 0.001$.

"Barents Sea West" group suggested by Asplund et al. (2004) is identical to the PCA group defined here as Atlantic Ocean and western Barents Sea, and the border between Asplund et al.'s (2004) "Kola Peninsula" and "White Sea" groups locates in the same area as the border between the Kola Peninsula and western White Sea groups of this study. The only exception is that in the present study, Asplund et al.'s (2004) "White Sea" group was divided into two separate groups whereby the western White Sea group consisted mostly of populations from the western coast of the White Sea, while the eastern Barents Sea group mainly included populations from Archangelsk. The fact that nuclear markers also divided the populations into separate groups indicates that the genetic structuring obtained with mtDNA haplotypes (Asplund et al. 2004) is not due to sex-biased dispersal or stronger drift in mtDNA. Most likely, these groups reflect the colonization history of the area, the signal of which should in theory slowly disappear unless the observed barriers are maintained by natural selection. However, further study is required before the relative roles of natural selection and phylogeographic history can be determined. In addition, it would be worthwhile to examine the temporal stability of the observed groups by sampling the same populations again. However, as most of the population samples assessed in this study were collected between 2000 and 2002 and the samples were a mixture of parr of different ages, it is highly unlikely that the observed population structuring is an artifact of sampling the populations at different time points.

Despite the clear boundaries observed between the groups, the AMOVA indicated that only a low proportion of genetic variation could be explained by differences among the groups. The results based on two different groupings were very similar, although the percentage of variation among groups based on the PCA was almost 20% higher (3.1%) than among groups based on their geographical location (2.6%). These numbers are much lower than the proportion of mtDNA haplotype variation among the three groups in Asplund et al. (2004) (22.1%). This result is characteristic of the highly variable microsatellite loci, and typically, the percentage of among-group variation is higher in studies based on mtDNA or allozyme variation. For example, in their study of allozyme variation in Atlantic salmon from the Baltic Sea, Koljonen et al. (1999) found that almost three times as much molecular variation was attributable to differences among Baltic salmon stock groups than did Säisä et al. (2005) in an investigation of similar populations applying microsatellites (15% versus 5.6%, respectively). Likewise, according to Verspoor et al. (1999), 43.9% of the mtDNA variation was due to differences between the Atlantic and Baltic regions, while in Tonteri et al. (2007), only 4.9% of the microsatellite variation stemmed from differences among the same regions. Here, population differentiation in general was slightly lower (global $F_{ST} = 0.07$) than in previous microsatellite studies on anadromous Atlantic salmon (global $F_{ST} = 0.11$ and 0.12: Säisä et al. 2005 and Tonteri et al. 2005, respectively), probably due to the smaller geographic scale and absence of Baltic populations from this study. The Baltic populations have been noted to be clearly distinguishable from the Atlantic populations in several studies (e.g., Nilsson et al. 2001, Säisä et al. 2005; Tonteri et al. 2005), and this is the most plausible cause for the higher global F_{ST} estimates in studies including both Baltic and Atlantic populations.

The low level of among-group variation observed in the AMOVA probably is also reflected in the moderate individual assignment efficiencies seeing that assignment success is affected by the level of population differentiation (Cornuet et al. 1999). Since the number of populations sampled was large and the geographical distances between populations were often very short, the number of loci applied seems not to be adequate for highly accurate identification of an individual's population of origin. A common trend seems to be that misassigned individuals were often assigned to a nearby population (e.g., from Lihodeevka to Pulonga Kola or vice versa) or to a population for which divergence from the source population was low (e.g., from Yapoma to Babya). In a few cases, the low success was most likely due to too low a number of sampled individuals (Reisa and Unja). Indeed, Kalinowski (2005) has shown by simulation that when highly polymorphic loci, such as microsatellites, are employed and the estimate of F_{ST} is less than 0.01, at least 100 individual should be sampled, while when F_{ST} is greater than 0.05, a sample of 20 individuals should suffice. Thus, to achieve a higher assignment success, it is advisable to increase the number of individuals in some populations and also the number of loci genotyped, as previous studies have shown that when F_{ST} is between 0.05 and 0.1, the best result is obtained with 20–30 loci (Cornuet et al. 1999). Here, the pairwise estimates of population divergence varied from 0.001 to 0.221 with a median of 0.064, implying that highly accurate individual assignment to population of origin would require additional loci and in some cases additional samples or, alternatively, the selection of more diagnostic loci (Banks et al. 2003; Rosenberg 2005). Nevertheless, the success rate for identifying the region of origin, as opposed to the specific population of origin, was considerably higher (87%–96%), indicating that the data were sufficient for relatively accurate assignment at this slightly broader scale. Therefore, the data obtained here can be used to differentiate individuals caught in offshore fisheries at a regional level (i.e., northeast Atlantic Ocean and western Barents Sea, eastern and southern Kola Peninsula, western White Sea, and eastern White and Barents seas) with a relatively high degree of accuracy.

Table 4. Number of individuals assigned to different populations based on their multilocus microsatellite genotype.

	Assigned to:																
	Atlantic and western Barents Sea											Kola Peninsula					
Origin	Dee	Sey	Rei	Ten	Tit	ZaL	Ura	Tul	Kola	Dro	Yok	Kac	Leb	Pac	Dan	Bab	Lib
Dee	**26**	2		3									1				
Sey	2	**28**	1		1				1		1						
Rei		1	**4**	2		1	3					1					
Ten				**19**	3	1	5	3	2		2	2	1	1			
Tit			2	4	**19**	3	1	1	1	1		1	3			2	
ZaL				3	5	**28**	5	1	1								
Ura			1	3	2	1	**28**	1	1	1	1						2
Tul				1	1		3	**30**	8			1	2			1	1
Kola	1			1		1	2	1	**26**			1	1		1	2	1
Dro				1			1		1	**34**	1		1				
Yok				1	1	1	1		2	1	**23**	4	1		1	1	1
Kac				2	1			1		1	4	**11**	2	2		3	4
Leb									1			2	**21**	3		5	4
Pac					1			1				6	8	**19**			2
Dan			1			1			1				2		**20**	5	1
Bab					1				1			3	3		4	**7**	3
Lib									1				3	1		5	**16**
PuK												1	4	2	2	10	7
Yog				2					1			3	1		3	4	5
Kit							1						3		3	2	2
Yap					1		1					2	3		1	5	4
Umb									2			2				3	1
Kofs													1			1	
Meg															1	4	
Ole																	1
Pil																	
Nil																	
PuW																	
Pon												1				1	2
Sum					1												
Esn					1				2								1
Pad																	
Piz																	
Unj														1			
Overall																	
Atlantic and western Barents Sea											**330**						
Kola Peninsula											28						
Western White Sea											0						
Eastern Barents Sea											4						

Note: Correct assignments are in boldface and assignments within a PCA group are shaded.

The above findings also help to clarify the current view of how Atlantic salmon colonized northwest Russia after the last glaciation period ended. The clear separation of the populations into northeastern Atlantic Ocean and western Barents Sea versus White and eastern Barents Sea populations implies that these regions were colonized from different refugia. Western immigration into the western Barents Sea has been suggested in earlier studies (e.g., Asplund et al. 2004; Makhrov et al. 2005; Tonteri et al. 2005) and is supported by the above. This is further corroborated by allele frequencies at locus *Ssa197* in which large alleles (293–329 bp) were found among 9 of the 11 populations forming the Atlantic Ocean and western Barents Sea group but not among populations farther to the east (supplementary Appendix S2).[3] Similarly, large alleles at locus *Ssa197* were only found among eastern Atlantic Ocean and Barents Sea populations and not in Baltic or White Sea populations by Säisä et al. (2005). An Atlantic influence in the western Barents Sea area has previously been suggested on numerous occasions: northeastern Atlantic mtDNA haplotypes have been found from the western Barents Sea in several studies (e.g., Nilsson et al. 2001; Asplund et al. 2004; Makhrov et al. 2005), and by using microsatellites as genetic markers, Tonteri et al. (2005) grouped populations from Dee, Teno, and Tuloma together. In addition, allozyme variation at the *ESTD** locus suggests immigration also from the western Atlantic Ocean, as the western Atlantic **80* allele has been found at several locations in the area (Kazakov and Titov 1991; Makhrov et al. 2005; Tonteri et al. 2005). This wealth of evidence thus implies that the western Barents Sea was colonized from the west rather than from the east after the last glaciation or that western lineages have at least contributed considerably to the genetic composition of contemporary Atlantic salmon populations from the area. The White and eastern Barents seas in turn were most likely colonized from the east as suggested before (Asplund et al. 2004; Tonteri et al. 2005) and supported by the current observation of distinctiveness of the populations from the region. Moreover, the absence of large alleles at *Ssa197* in all of the White and eastern Barents Sea populations suggests that eastern Atlantic immigration has not arrived to the area.

Although significant IBD was found when all of the

							Western White Sea					Eastern Barents Sea						
PuK	Yug	Kit	Yap	Umb	Kolv	Meg	Ole	Pil	Nil	PuW	Pon	Sum	Emt	Pad	Piz	Unj	Total N	Correctly assigned (%)
																	32	81.3
																	27	77.8
1														1			14	28.6
	3	1															43	44.2
	1											1					40	47.5
		1															40	70.0
																	41	68.3
	1												1				51	60.8
2		1											1				42	61.9
											1						40	85.0
			2														40	57.5
1	**5**		**1**	**1**													41	26.8
2	**3**	**3**	**1**		**1**		1										47	44.7
	2			**1**													38	50.0
4	**3**	**2**	**2**	**1**									1				44	45.5
7	**6**	**4**	**3**	**2**							1		1				46	15.2
9	**6**		**5**	**1**													47	34.0
10	**2**	**1**	**3**		**1**	**1**							2				47	21.3
3	**26**	**4**	**2**	**1**			1										56	46.4
4	**4**	**12**	**3**	**2**			1										37	32.4
3	**1**	**3**	**15**			**1**											40	37.5
1	**1**	**1**	**1**	**33**							2						47	70.2
1	**1**			**1**	**36**												41	87.8
1	**3**		**1**			**30**							3				43	69.8
	1	1					**31**										34	91.2
				1				**32**			**2**						35	91.4
									42								42	100
										43							45	100
		1									**45**						50	90.0
												40					41	97.6
													37	**1**			42	88.1
													1	**47**			48	97.9
	1														**21**		22	95.5
															2	**8**	11	72.7
						47					1					4	410	87.3
						533					6					7	574	92.9
						9					195					0	204	95.6
						3					0					157	164	95.7

studied populations were included in the analysis, groupwise significant IBD was observed only among populations forming the Atlantic Ocean and western Barents Sea and Kola Peninsula groups, while in the other two groups, no sign of IBD was detected. We found substantial variation in the IBD regression slope when populations separated by 100 km or less were considered. This variation cannot be due to population bottlenecks, as none were detected in the present study. However, the reason for the variation might be the low number of population pairs included in the calculation of the slope at short geographical distances, and thus, the slope value could vary at random. The slope was observed to decrease at geographical distances between 100 and 180 km after which a plateau close to zero was reached. A similar pattern of IBD fading has been detected in other studies (Ehrich and Stenseth 2001; Castric and Bernatchez 2003) and various explanations proposed. Fore example, Castric and Bernatchez (2003) suggested that such a pattern could be observed if the upper limit of F_{ST} has been reached. As Hedrick (1999, 2005) pointed out, loci with high within-population heterozygosity, such as microsatellites, may give underestimates of F_{ST}, which could thus plateau at large geographic distances. Second, the IBD pattern could fade if populations are in nonequilibrium (Ehrich and Stenseth 2001; Castric and Bernatchez 2003). If insufficient time has passed since an area has been colonized by a species, the populations may exhibit a nonequilibrium situation. In such a case, only nearby populations are expected to show IBD and more distant ones not (Slatkin 1993). Third, the role of mutations should be considered as well, as mutations arising randomly in space might weaken the relationship between genetic and geographical distance (Castric and Bernatchez 2003).

In this study, it is unlikely that the fading of IBD is due to F_{ST} reaching its upper limit. The same loci as utilized here have also been employed to study landlocked Atlantic salmon populations of northwest Russia and even higher estimates of F_{ST} were obtained (C.R. Primmer, M.Yu. Ozerov, A.Je. Veselov, and J. Lumme, unpublished data). A more likely explanation is that the studied populations exhibit a nonequilibrium situation, as the whole studied area was covered by a massive ice sheet during the last glacial maximum

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	Assigned to																
	Atlantic and western Barents Sea											Kola Peninsula					
Origin	Dee	Sey	Rei	Ten	Tit	Zol	Uts	Tul	Kola	Dro	Yok	Kac	Leb	Pac	Dan	Bab	Lih
Dee	**26**	2		3									1				
Sey	2	**21**	1		1				1		1						
Rei		1	**4**	2		1	3					1					
Ten				**19**	3	1	5	3	2		2	2	1	1			
Tit			2	4	**19**	3	1	1	1	1		1	1			2	
Zol				3	3	**28**	3	1	1								
Uts			1	3	2	1	**28**	1	1	1	1						2
Tul				1	1		3	**31**	8			1	2			1	1
Kola	1			1		1	2	1	**26**			1	1		1	2	1
Dro				1			1		1	**34**	1		1				
Yok				1	1	1	1		2	1	**23**	4	1		1	1	1
Kac				2	1			1		1	4	**11**	2	2		3	4
Leb									1			2	**21**	3		5	4
Pac					1			1				4	8	**19**			2
Dan			1			1			1				2		**20**	5	1
Bab					1				1			3	3		4	**7**	3
Lih									1				3	1		5	**16**
PnK												1	4	2	2	10	7
Vrz				2					1			3	1		3	4	5
Kit							1						3		3	2	2
Vap					1		1					2	3		1	5	4
Umb									2			2				3	1
Kolv													1			1	
Mcg															1	4	
Olc																	1
Pil																	
Nil																	
PaW																	
Pon												1				1	2
Sum					1												
Unn					1				2								1
Pad																	
Pie																	
Ung														1			
Overall																	
Atlantic and western Barents Sea											**358**						
Kola Peninsula											28						
Western White Sea											0						
Eastern Barents Sea											4						

Note: Correct assignments are in boldface and assignments within a PCA group are shaded.

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							Western White Sea					Eastern Barents Sea						
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																	32	81.3
																	27	77.8
1														1			14	28.6
	3	1															43	44.2
	1											1					40	47.5
		1															40	70.0
																	41	68.3
	1												1				51	60.8
2		1											1				42	61.9
											1						40	85.0
			2														40	57.5
1	5		1	1													41	26.8
2	3	3	1		1		1										47	44.7
	2			1													58	50.0
4	3	2	2	1									1				44	45.5
7	6	4	3	2							1		1				46	15.2
9	6		5	1													47	34.0
10	2	1	1		1	1							2				47	21.3
3	**26**	4	2	1			1										56	46.4
8	4	**12**	1	2			1										37	32.4
3	1	3	**15**			1											40	37.5
1	1	1	1	**33**							2						47	70.2
1	1			1	**36**												41	87.8
1	3		1			**30**							1				43	69.8
	1	1					**31**										34	91.2
				1				**32**			2						35	91.4
									42								42	100
										43							43	100
		1									**45**						50	90.0
												40					41	97.6
													37	1			42	88.1
													1	**47**			48	97.9
	1														**21**		22	95.5
															2	**8**	11	72.7
						47					1					4	410	87.3
						533					6					7	574	92.9
						9					**195**					0	204	95.6
						3					4					**157**	164	95.7

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Table 5. Summary of the average population genetic diversity and divergence characteristics, occurrence of a significant signal of isolation by distance (IBD) with a region, and average population-level individual assignment success within the four subregions identified in the study.

Subregion	Genetic diversity (A_r, H_e)	Genetic divergence (F_{ST})	Significant IBD?	Average assignment success (%)
Atlantic Ocean and western Barents Sea	7.3, 0.73	0.032	Yes	62
Kola Peninsula	7.1, 0.71	0.022	Yes	45
Western White Sea	5.1, 0.65	0.101	No	95
Eastern Barents Sea	5.3, 0.64	0.108	No	90

approximately 25 000 – 15 000 years ago (e.g., Svendsen et al. 2004). The Archangelsk area was freed of ice approximately 13 000 years ago and the White Sea and most of the Kola Peninsula approximately 11 500 – 12 000 years ago (Svendsen et al. 2004). It may thus be that Atlantic salmon from the White and Barents seas are still going through a transitory phase towards equilibrium and IBD cannot be detected at large geographical distances.

Given the population genetic structuring estimated for the region, which populations should be prioritized for conservation? The results clearly indicate that conservation of populations from each of the observed clusters is recommended. Inclusion of just two populations from each group would retain considerably more of the entire region's genetic diversity than conservation of all of the populations from a single group. This indicates that the four groups with different postglacial histories identified here can be considered as a good starting point for defining four distinct management units. Considering the separate groups, Russian Atlantic salmon populations from the western Barents Sea group retained considerably less genetic diversity (23.5%) than populations forming the other three groups (34.4%, 32.7%, and 31.9%, respectively). Within the Atlantic Ocean and western Barents Sea group, populations Drozdovka, Zapadnaya Litsa, and Yokanga seemed to preserve the most genetic diversity (17.6%), and among the Kola Peninsula group, Kolvitsa, Megra, and Ponoi Pacha together retained a similar proportion of diversity (18.0%). In the western White and eastern Barents Sea groups, more variation was maintained already in two populations: Nilma and Pulonga White Sea 19.5% and Suma and Pechora Unja 19.7%. Thus, compared with the western and eastern White and eastern Barents seas, it seems that more populations from the western Barents Sea and Kola Peninsula groups need to be conserved to maintain diversity in these areas. This is not surprising given the levels of genetic divergence in the respective groups and the fact that Conserve 1.4b diversity contributions are solely based on genetic divergence: as the average level of genetic divergence within the western Barents Sea and Kola Peninsula regions is lower, preservation of a larger number of populations is required to conserve a similar proportion of the total diversity compared with other regions.

Contrib 1.02 recognized partly the same populations as Conserve 1.4b to have a high conservation value. Titovka and Tuloma in the Atlantic Ocean and western Barents Sea group, Varzuga Yapoma in the Kola Peninsula group, and Pila in the western White Sea group had the highest net contributions to allelic richness in addition to Zapadnaya Litsa, Kolvitsa, Megra, Nilma, and Pulonga White Sea already pointed out by the other method. In the eastern Barents Sea group, the three populations with the highest GD %, namely Suma, Pechora Unja, and Severnaja Dvina Padoma, all had negative net contributions to total allelic richness. However, the divergence components of these three were very high, thus suggesting them to be more valuable for conservation than Severnaja Dvina Emtsa, which had the highest total contribution to allelic richness but low C_S^r and C_D^r components. This emphasizes the importance of considering the C_S^r and C_D^r components when interpreting the results of Contrib 1.02 and not just the total contribution of each population. More importantly, it is advisable to base management plans on several prioritizing methods, as different methods pinpoint different populations worthy of conservation.

Even though no signs of recent reductions in the effective size of any of the studied populations were observed, the low allelic richness and high population divergence together with no indication of IBD among the western White Sea and eastern White and Barents Sea populations might nevertheless imply that the effects of genetic drift in these populations may be strong, thus possibly questioning the prioritization of populations from these groups for conservation. It would therefore be especially important to investigate the temporal stability of these populations to shed more light on the effects of genetic drift. In the event that their genetic structure is temporally stable, they may in fact be good candidates for having evolved local adaptations (discussed in detail by Adkison 1995) and thus be of high priority for conservation.

In addition to analyzing population divergence with neutral markers, future analyses of quantitative trait differentiation may also be useful for inferring the relative roles of random genetic drift and natural selection in explaining the observed population genetic structure of Atlantic salmon of northwest Russia. Furthermore, comparisons of neutral marker and quantitative trait differentiation (F_{ST} versus Q_{ST}) can also be useful from a conservation perspective, as such comparisons can reveal adaptive divergence even when neutral genetic divergence is very low (Leinonen et al. 2008). With such a strategy, management units not identifiable by neutral markers alone can also be defined. As pointed out by Crandall et al. (2000) and Fraser and Bernatchez (2001), basing conservation decisions on neutral genetic variation alone is not always the optimal solution, but inclusion of ecological data such as life history, morphological, and quantitative traits might help to find the optimal solution for preserving the evolutionary potential of a species. However, in the absence of such comprehensive information, population structure based on assumedly neutral markers such as microsatellites nevertheless provides a good starting point for conservation prioritization.

In summary, the four groups with different postglacial histories identified here can be considered as a good starting point for defining four distinct management units. These four groups may indeed require different management strategies, as two distinct patterns of genetic diversity and divergence characteristics were detected (Table 5). Populations within the Atlantic Ocean and western Barents Sea and Kola Peninsula groups were genetically more diverse and less diverged than populations in the western White and eastern Barents Sea groups, with an IBD signal only being observed in the former two groups (Table 5). This implies that the relative importance of migration versus genetic drift in defining population structure in these subregions is likely different, with migration having a greater influence in the Atlantic Ocean and western Barents Sea and Kola Peninsula region and genetic drift being more important in the western White and eastern Barents seas.

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Appendix A

Table A1 appears on the following page.

Table A1. Pairwise genetic distances (F_{ST}) (below the diagonal) and geographical distances (km) (above the diagonal).

	Dee	Sey	Rei	Ten	Tir	Zal.	Uta	Tul	Kola	Dro	Yok	Kac	Leb	Pac	Dan	Bab	Lib
Dee		924	1855	2198	2442	2432	2428	2549	2495	2635	2695	2792	3002	2971	2879	2931	2939
Sey	0.041		1053	1397	1641	1631	1627	1748	1694	1834	1894	1991	2201	2170	2078	2130	2138
Rei	0.055	0.052		406	650	640	636	757	703	843	903	1000	1210	1179	1087	1139	1147
Ten	0.045	0.052	0.011		315	305	301	422	368	514	574	667	877	846	754	806	814
Tir	0.043	0.041	0.019	0.009		54	77	205	151	310	370	463	673	642	550	602	610
Zal.	0.065	0.059	0.024	0.015	0.020		60	189	135	294	354	447	657	626	534	586	594
Uta	0.058	0.060	0.009	0.006	0.015	0.014		166	112	272	332	425	635	604	512	564	572
Tul	0.044	0.056	0.036	0.018	0.015	0.025	0.018		75	356	416	509	719	688	596	648	656
Kola	0.044	0.055	0.038	0.021	0.009	0.029	0.020	0.005		302	362	455	665	634	542	594	602
Dro	0.063	0.068	0.039	0.026	0.029	0.038	0.033	0.039	0.034		67	164	374	343	251	303	311
Yok	0.060	0.071	0.036	0.020	0.022	0.037	0.021	0.028	0.026	0.032		119	329	298	206	258	266
Kac	[illegible]	[illegible]	[illegible]	0.026	0.029	0.042	0.030	0.025	0.014	0.031	0.014		210	179	87	139	147
Leb	0.072	0.076	0.059	0.053	0.033	0.063	0.052	0.044	0.035	0.059	0.038	0.013		30	185	237	245
Pac	0.072	0.075	0.066	0.057	0.035	0.063	0.059	0.047	0.038	0.063	0.038	0.021	0.015		154	206	214
Dan	0.061	0.078	0.047	0.040	0.035	0.065	0.044	0.040	0.031	0.046	0.037	0.014	0.016	0.012		52	60
Bab	0.060	0.075	0.042	0.036	0.032	0.057	0.035	0.026	0.022	0.047	0.028	0.007	0.017	0.031	0.003		8
Lib	0.067	0.085	0.058	0.052	0.040	0.070	0.050	0.049	0.030	0.061	0.037	0.011	0.014	0.028	0.010	0.004	
PaK	0.068	0.083	0.051	0.042	0.033	0.064	0.042	0.033	0.028	0.051	0.037	0.009	0.017	0.033	0.008	0.001	0.008
Yug	0.067	0.080	0.041	0.032	0.034	0.054	0.034	0.034	0.032	0.052	0.027	0.013	0.021	0.041	0.011	0.005	0.013
Kit	0.066	0.086	0.048	0.040	0.035	0.061	0.042	0.032	0.029	0.060	0.036	0.010	0.018	0.031	0.008	0.003	0.010
Yap	0.052	0.077	0.051	0.038	0.030	0.056	0.039	0.028	0.024	0.047	0.029	0.010	0.022	0.035	0.015	0.010	0.012
Ole	0.119	0.130	0.102	0.092	0.084	0.113	0.094	0.087	0.081	0.114	0.076	0.064	0.075	0.083	0.061	0.051	0.058
Umb	0.084	0.085	0.062	0.061	0.051	0.073	0.057	0.048	0.039	0.074	0.054	0.024	0.029	0.044	0.026	0.020	0.017
Pil	0.119	0.149	0.115	0.106	0.094	0.122	0.108	0.094	0.094	0.108	0.086	0.074	0.075	0.095	0.079	0.072	0.074
Kolv	0.084	0.094	0.076	0.062	0.053	0.076	0.065	0.056	0.044	0.071	0.058	0.037	0.036	0.047	0.033	0.029	0.034
Nil	0.127	0.133	0.123	0.104	0.085	0.117	0.110	0.089	0.086	0.108	0.094	0.088	0.108	0.102	0.115	0.096	0.102
PaW	0.117	0.133	0.111	0.103	0.089	0.118	0.100	0.088	0.095	0.111	0.092	0.088	0.097	0.103	0.103	0.089	0.098
Pon	0.100	0.099	0.056	0.059	0.049	0.080	0.054	0.061	0.054	0.077	0.045	0.043	0.055	0.061	0.044	0.035	0.042
Sum	0.138	0.182	0.162	0.121	0.108	0.144	0.126	0.113	0.105	0.133	0.111	0.094	0.107	0.109	0.089	0.097	0.109
Emt	0.077	0.089	0.107	0.075	0.062	0.088	0.089	0.055	0.044	0.088	0.078	0.043	0.054	0.053	0.051	0.048	0.047
Pad	0.131	0.150	0.172	0.129	0.130	0.146	0.145	0.115	0.112	0.136	0.132	0.096	0.104	0.114	0.104	0.103	0.118
Meg	0.072	0.081	0.076	0.065	0.050	0.077	0.070	0.052	0.039	0.075	0.066	0.030	0.032	0.041	0.030	0.028	0.017
Piz	0.117	0.127	0.111	0.088	0.087	0.096	0.099	0.081	0.084	0.128	0.107	0.085	0.084	0.086	0.081	0.080	0.091
Unj	0.140	0.134	0.122	0.094	0.087	0.095	0.099	0.087	0.084	0.129	0.113	0.087	0.084	0.090	0.078	0.080	0.096

Note: Nonsignificant F_{ST} values are underlined ($P > 0.05$).

PuK	Yug	Kit	Yap	Ole	Umb	Pil	Kolv	Nil	PuW	Pon	Sum	Emt	Pad	Meg	Piz	Unj
2952	3013	3103	3151	3176	3247	3244	3318	3267	3260	3255	3261	3359	3589	2952	3719	4607
2151	2212	2302	2350	2375	2446	2443	2517	2466	2459	2454	2460	2558	2788	2151	2918	3806
1160	1221	1311	1359	1384	1455	1452	1526	1475	1468	1463	1469	1567	1797	1160	1927	2815
827	888	978	1026	1051	1122	1119	1193	1142	1135	1130	1136	1234	1464	827	1602	2490
623	684	774	822	847	918	915	989	938	931	926	932	1030	1260	623	1464	2352
607	668	758	806	831	902	899	973	922	915	910	916	1014	1244	607	1448	2336
585	646	736	784	809	880	877	951	900	893	888	894	992	1222	585	1426	2314
669	730	820	868	893	964	961	1035	984	977	972	978	1076	1306	669	1510	2398
615	676	766	814	839	910	907	981	930	923	918	924	1022	1252	615	1456	2344
324	385	475	523	548	619	616	690	639	632	627	633	731	961	324	1219	2107
279	340	430	478	503	574	571	645	594	587	582	588	686	916	279	1183	2071
160	221	311	359	384	455	452	526	475	468	463	469	567	797	160	1185	2073
258	319	409	457	482	553	550	624	573	566	561	567	665	898	262	1379	2267
227	288	378	426	451	522	519	593	542	535	530	536	635	868	232	1349	2237
73	134	224	272	297	368	365	439	388	381	376	382	481	714	81	1250	2138
21	82	172	220	245	316	313	387	336	329	324	330	433	666	73	1302	2190
13	74	164	212	237	308	305	379	328	321	316	322	426	659	74	1310	2198
	61	151	199	224	295	292	366	315	308	303	309	415	648	81	1323	2211
0.012		90	138	163	234	231	305	254	247	250	255	406	639	138	1384	2272
0.007	0.008		52	81	152	149	223	176	166	198	242	474	707	228	1474	2362
0.009	0.014	0.009		128	200	196	271	227	216	236	289	519	752	275	1522	2410
0.060	0.055	0.046	0.064		71	68	142	101	167	94	245	535	768	301	1547	2435
0.029	0.021	0.022	0.029	0.059		40	110	76	207	209	291	602	835	372	1618	2506
0.085	0.074	0.075	0.075	0.101	0.071		96	61	205	206	288	599	832	369	1615	2503
0.036	0.045	0.043	0.038	0.095	0.046	0.097		96	124	271	352	675	908	443	1689	2577
0.096	0.100	0.100	0.091	0.120	0.113	0.097	0.118		69	219	296	623	856	396	1638	2526
0.095	0.092	0.092	0.091	0.118	0.093	0.125	0.124	0.144		192	273	605	838	386	1631	2519
0.047	0.039	0.041	0.047	0.063	0.041	0.060	0.068	0.089	0.098		168	554	787	380	1626	2514
0.093	0.104	0.088	0.098	0.172	0.137	0.176	0.129	0.182	0.187	0.135		526	759	374	1632	2520
0.045	0.059	0.043	0.045	0.096	0.058	0.129	0.064	0.114	0.137	0.094	0.116		291	455	1737	2625
0.095	0.111	0.096	0.099	0.192	0.131	0.210	0.117	0.194	0.211	0.170	0.145	0.058		688	1970	2858
0.031	0.034	0.026	0.026	0.068	0.026	0.102	0.050	0.113	0.108	0.067	0.124	0.029	0.114		1319	2207
0.078	0.087	0.078	0.083	0.144	0.097	0.162	0.093	0.172	0.179	0.111	0.158	0.075	0.124	0.074		1080
0.078	0.082	0.072	0.087	0.142	0.096	0.182	0.087	0.182	0.198	0.119	0.148	0.065	0.116	0.075	0.054	

Freshwater and marine migration and survival of endangered Cultus Lake sockeye salmon (*Oncorhynchus nerka*) smolts using POST, a large-scale acoustic telemetry array

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Abstract: Freshwater and early marine migration and survival of endangered Cultus Lake sockeye (*Oncorhynchus nerka*) salmon were studied using the Pacific Ocean Shelf Tracking (POST) array. Smolts were acoustically tagged in 2004–2007, and their migration was recorded within the lower Fraser River and coastal southern British Columbia waters. Most smolts showed rapid directional movement (swimming speeds of $\sim$15–30 km·day^{-1}). Average exit time from the Fraser River was 4.0–5.6 days after release, and average residence time within the Strait of Georgia was 25.6–34.1 days. Most individuals migrated northward, generally close to the mainland coast. Survival rates, assessed using standard mark–recapture methods, were generally high during the downstream migration (50%–70%), except in 2005 when survival was <20%, possibly because of a late release. Marine survival rates were stable among years, between 10%–30% at a subarray sited 500 km away from the release site. Movement rates were similar to those of previously published work, but the POST array provided direct measurements of movement and estimates of survival and demonstrated the feasibility of establishing continental-scale acoustic arrays for management and conservation of marine species.

Résumé : Nous étudions la migration et la survie en eau douce et au début de la vie marine chez le saumon rouge (*Oncorhynchus nerka*) du lac Cultus, une forme menacée, à l'aide du réseau POST (« Pacific Ocean Shelf Tracking », surveillance de la plate-forme du Pacifique). Les saumoneaux ont été munis d'étiquettes acoustiques en 2004–2007 et leur migration enregistrée dans le Fraser inférieur et les eaux côtières de la Colombie-Britannique. La plupart des saumoneaux font des déplacements directionnels rapides (vitesses de nage $\sim$15–30 km·jour^{-1}). Le temps moyen pour quitter le Fraser est de 4,0–5,6 jours après la libération et le temps moyen de résidence dans le détroit de Géorgie est de 25,6–34,1 jours. La plupart des individus migrent vers le nord, généralement près des côtes du continent. Les taux de survie, déterminés par des méthodes classiques de marquage et de recapture, sont généralement élevés durant la migration vers l'aval (50–70 %) excepté en 2005 où la survie était <20%, probablement à cause d'une libération tardive. Les taux de survie en mer sont stables au cours des années, entre 10–30 % dans un sous-réseau situé à 500 km du point de libération. Les taux de déplacement sont semblables à ceux mentionnés dans des travaux antérieurs publiés, mais le réseau POST fournit des mesures directes des déplacements et des estimations de la survie. Notre étude démontre qu'il est possible d'établir des réseaux acoustiques à l'échelle continentale pour la gestion et la conservation des espèces marines.

[Traduit par la Rédaction]

Introduction

The management and conservation of salmon populations would be aided by a better understanding of the marine life-history phase (e.g., McKinnell et al. 2001; English et al. 2008; Lacroix 2008). The early marine stage is poorly studied relative to both the initial freshwater life-history phases (egg to fry, fry to smolt) and the very late marine and freshwater life-history phases of returning adults (e.g.,

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Groot and Margolis 1991). Although there are many estimates of overall survival for Pacific salmon (*Oncorhynchus* spp.) stocks based on outmigrating smolt numbers and the subsequent level of adult return some years later, it has not been possible to directly study survival at discrete periods during the marine portion of their life history. It is therefore difficult to pinpoint where major periods of mortality occur or understand their cause. Such information is necessary for understanding population dynamics and for making informed decisions for assisting the rebuilding of threatened and endangered populations of salmon.

The development and continued miniaturization of uniquely coded acoustic tags in the last decade holds the promise of allowing major advances in marine research (Voegeli et al. 1998, 2001; Heupel et al. 2006). Unlike radio waves, sound transmits effectively in both fresh and saline waters. Acoustic receivers can be arranged in line arrays or "curtains" (e.g., Lacroix and Voegeli 2000; Lacroix et al. 2005) placed in selected locations along potential migratory routes. In this way, it is possible both to track individuals seamlessly between the fresh and marine environments and to partition survival to understand where episodes of mortality occur.

This technology has formed the basis of the Pacific Ocean Shelf Tracking Project (POST) for tracking both juvenile (Welch et al. 2002, 2004; Melnychuk et al. 2007) and adult (e.g., Cooke et al. 2008) salmon, as well as other species (Welch et al. 2006; Lindley et al. 2008). The POST array is intended as a permanent continental-scale telemetry research platform for the entire west coast of North America, including major rivers. The component listening "curtains" (or subarrays) use Vemco VR-2 or VR-3 passive acoustic receivers (Amirix Systems Inc., Halifax, Nova Scotia) in precise geometries to enable a nearly complete census of acoustically tagged salmon as they cross each successive curtain. Although centered along the shelf in southern British Columbia waters, the array currently extends almost 900 km up the Columbia–Snake rivers in the United States and spans over 2500 km in geographic extent from the Columbia River to Graves Harbor, southeast Alaska (Haggan et al. 2009).

In this study, we used the POST array to study the migratory behaviour and survival of Cultus Lake sockeye (*Oncorhynchus nerka*) smolts over the 4-year period from 2004 to 2007. This sockeye population is genetically unique and has been studied since the 1920s (Ricker 2006). Between the 1920s and the 1960s, the number of returning adult Cultus sockeye averaged around 20 000. However, a rapid decline in the number of adult sockeye returning to spawn in the 1990s reduced the population to fewer than 100 (decreasing by 92% between 1991 and 2002; English et al. 2008), triggering a recommendation for an emergency listing of the population as endangered by the Committee on the Status of Endangered Wildlife in Canada in 2002 (Committee on the Status of Endangered Wildlife in Canada [COSEWIC] 2003). Although the federal government accepted the evidence, legal listing as endangered was not made because of the impact that the decision would have on commercial fisheries of other stocks of Fraser sockeye. (Cultus Lake sockeye are incidentally harvested during the late-run Fraser River sockeye fishery and fisheries closures would lead to economic losses estimated to be on the order of $200 million; Government of Canada 2005.)

What is unclear is why marine survival decreased dramatically in the 1990s. COSEWIC (2003) listed the three primary causes of sockeye decline as overfishing and incidental harvest, high prespawning mortality, and low marine survival. Poor marine survival seems to be a feature shared by a number of salmonid stocks and other species in south and central coastal British Columbia waters (English et al. 2008), and the cause clearly warrants further investigation. The Cultus Sockeye Recovery Team states that "survival and distribution of Cultus sockeye in the Fraser River and nearshore marine areas have not been documented, thus resulting in a knowledge gap for this specific stock" (Cultus Sockeye Recovery Team 2005). It was thus of interest to explore how tracking migrating Cultus Lake sockeye smolts using the POST array might help to fill gaps in our understanding of where possible survival "bottlenecks" might occur.

The objectives of our study were to estimate survival and migratory behaviour of Cultus Lake sockeye smolts within the Fraser River and along their initial coastal migration. This work builds on our existing understanding from earlier investigations into the migration of Fraser River sockeye salmon (Groot and Cooke 1987; Groot and Quinn 1987; Groot et al. 1989).

Materials and methods

Study site

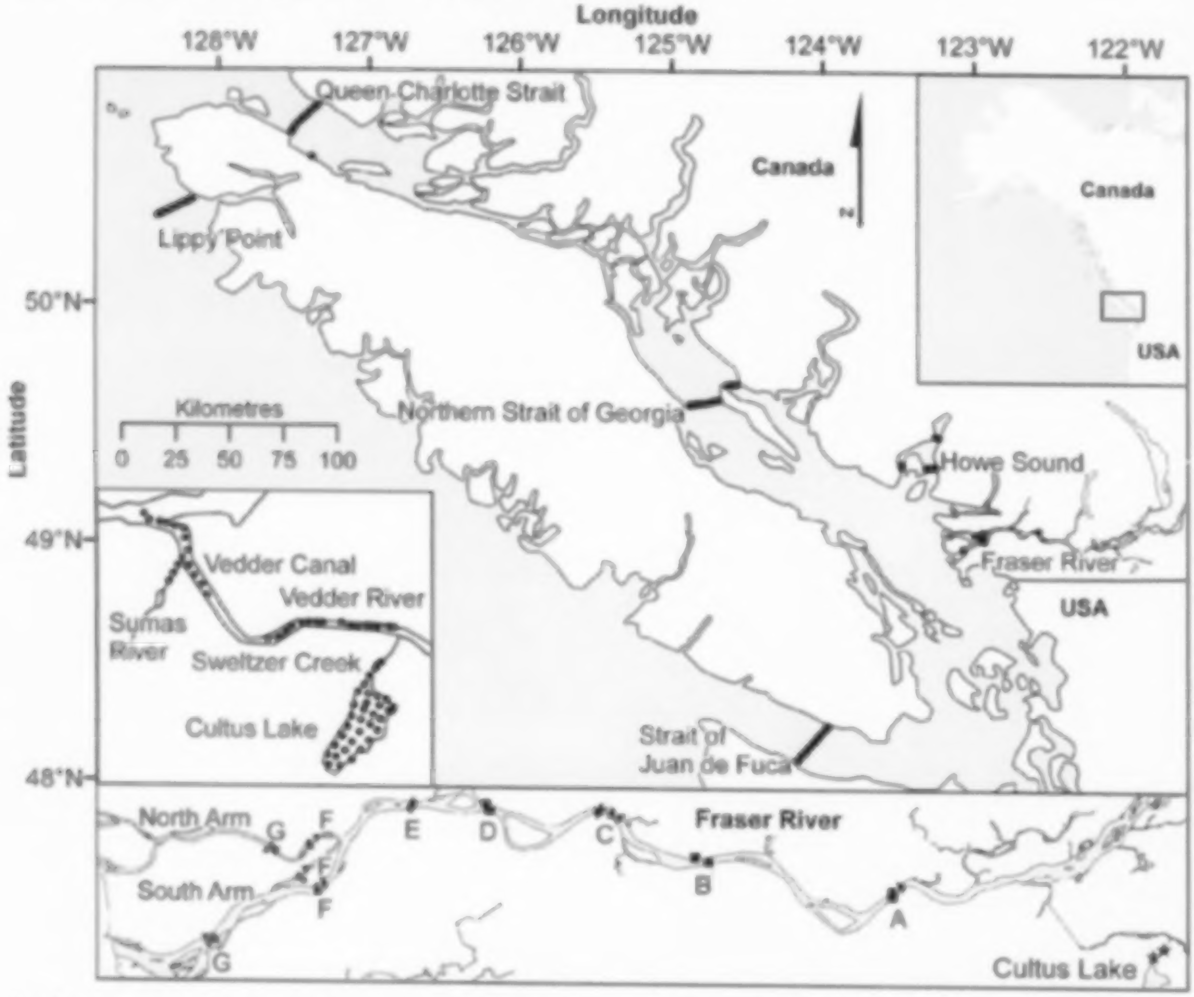
Cultus Lake drains into the lower Fraser River via Sweltzer Creek, Vedder River, and Sumas River (Fig. 1). The total distance from the release site at the lake outflow to the mouth of the Fraser River is 100 km. Mean annual discharge of the Fraser River, which drains into the Strait of Georgia, is 3600 $m^3 \cdot s^{-1}$, with highest flows peaking in late June. Exit routes to the Pacific Ocean from the Strait of Georgia include Johnstone Strait and Queen Charlotte Strait to the north and the Strait of Juan de Fuca to the south (Fig. 1).

Smolt release groups

Hatchery-reared 1.5-year-old sockeye salmon smolts were surgically implanted with acoustic transmitters prior to release in 2004–2007. All smolts were from Cultus Lake stock. Those tagged in 2004, 2005, and 2007 were reared from wild adult returns. Those tagged in 2006 were from more complex origins because only 10 females were available for broodstock, which were taken from a total return of only 60 adults in the 2004 brood year. These smolts were produced from wild × wild parents (85%) or a mixture of hatchery-produced, naturally returning parents (one or both parents; 15%). Some of the 2006 matings also used cryopreserved sperm taken from wild adult males in 2002.

In 2004 (n = 100), sockeye smolts were reared at Rosewall Creek Hatchery on Vancouver Island, tagged, and then transported by helicopter to Cultus Lake for release. In 2005 (n = 376), 2006 (n = 200), and 2007 (n = 200), smolts were tagged at Inch Creek Hatchery (located near Cultus Lake) and then transported by truck to Cultus Lake for release. In all years except 2005, tagged fish were released in Sweltzer Creek at the outflow from Cultus Lake. A fence in Sweltzer

Fig. 1. The southern British Columbia components of the POST array. The thick black lines indicate POST subarrays, which are formed by a linearly arranged sequence of independent receivers. Inserts provide details of the POST lines within the Fraser River and Cultus Lake and its outlets, along with mobile sampling locations in those areas (●). The bottom panel shows the array deployment within the Fraser River over the 4 years of the study. Subarray locations in the Fraser River differed between years and are identified by symbols and letters: ■ (A, B, D), 2004; ◆ (C, E), 2005; ▲ (C, F, G), 2006; ▼ (A, C, F, G), 2007.


Longitude
128°W
127°W
126°W
125°W
124°W
123°W
122°W
Latitude
50°N
49°N
48°N
Queen Charlotte Strait
Lippy Point
Canada
N
Canada
USA
Kilometres
0 25 50 75 100
Northern Strait of Georgia
Howe Sound
Fraser River
USA
Vedder Canal
Vedder River
Sumas River
Sweltzer Creek
Cultus Lake
Strait of Juan de Fuca
North Arm
South Arm
G
F
F
F
E
D
C
B
A
G
Fraser River
Cultus Lake

Creek upstream of the release site was in place each year until about 1 June to reduce the frequency of upstream movement into the lake. In 2005, fish were released in June after the fence had been removed, and therefore, they were released approximately 500 m downstream of the standard release site.

Average fork length (FL) of all Cultus Lake hatchery smolts tagged over the 4 years of the study ranged from 159 to 189 mm (Table 1). It should be noted that these hatchery-reared smolts were considerably larger than typical wild smolts (~100 mm), but currently available acoustic tags are too large for use in wild sockeye smolts.

Tag characteristics

Tags were programmed to transmit their acoustic codes at 69 kHz with a random time interval of 30–90 s between successive transmissions (average 60 s). In 2004, we implanted Vemco V9-6L tags, which had projected life spans of >4 months. In 2005, we used a combination of V9-6L, V9-1L, and V9-2L tags (Table 1). The latter two models have an extended battery life because of their larger size. We programmed a "sleep" period that began on 22 July 2005 (V9-1L) or 1 December 2005 (V9-2L); during this time, acoustic transmission was terminated to reserve power for the 2007 return migration of any surviving adults. These tags were programmed to reactivate and begin transmission again on 30 June 2007, prior to the historic arrival time in the Strait of Georgia. The tags had sufficient projected reserve battery power to allow transmission to continue until at least 15 September 2007, after the time of expected adult entry into the Fraser River. The larger tags were implanted into larger smolts (see Table 1). In 2006 and 2007, we used

Table 1. Summary of Cultus Lake sockeye smolts tagged and released.[a]

Year	Tag type[b]	Release date (UTC)	Number	Fork length (mm)	
				Average	SD
2004	V9-6L	04 May 2004 (0330)	100	178	13
2005	V9-1L	09 June 2005 (0300)	92	172	5
	V9-2L	09 June 2005 (0300)	188	188	8
	V9-6L	09 June 2005 (0300)	96	159	5
2006	V9-1L	21 April 2006 (0100)	200	178	9
2007	V9-1L	16 May 2007 (2200)	200	189	8

Note: UTC, coordinated universal time; SD, standard deviation.

[a] Detailed data are available in the POST database (Pacific Ocean Shelf Tracking Project 2008).

[b] Tag dimensions: V9-6L tags are 21 mm × 9 mm and weigh 2.9 g in air; V9-1L tags are 24 × 9 mm and weigh 3.6 g in air; and V9-2L tags are 29 mm × 9 mm and weigh 4.7 g in air.

all V9-1L tags with 41–45 days of active transmission after release until their programmed sleep periods began on 1–4 June 2006 or 27–28 June 2007. The 2006 tags were programmed to reactivate between 18 April and 4 July 2008, whereas the 2007 tags should reactivate on 26 July 2009.

Tags ranged in length between 10.0% and 16.7% of body length (mean ratios: V9-6L, 12.5%; V9-1L, 13.3%; V9-2L, 15.5%) and in mass between 2.4% and 9.6% of body mass (mean ratios: V9-6L, 5.5%; V9-1L, 5.1%; V9-2L, 5.9%). Tag–body size ratios of nearly all fish were below recommended limits for juvenile salmonids of 16% of length and 8% of weight (Lacroix et al. 2004).

Surgical techniques

Acoustic tags were surgically implanted into smolts following standard protocols (Welch et al. 2007). Smolts were sedated with 1 ppm metomidate HCl (Aquacalm; Syndel Laboratories Ltd., Qualicum Beach, B.C.) for a minimum of 10 min, and then 3–4 min prior to surgery, they were brought under full anaesthesia using 70 ppm Tricaine methanesulphonate (MS-222) mixed with a 140 ppm $NaHCO_3$ buffer. Gills were continuously irrigated during surgery using a purpose-built surgical table with recirculating water bath containing 50 ppm MS-222 (100 ppm $NaHCO_3$) flowing into the mouth. A tag was inserted into the abdominal cavity through a midventral line incision, which was closed using two to three polydioxanone monofilament sutures. Fish were then transferred to a recovery tank. Water baths were continually monitored for temperature and dissolved oxygen. All water baths were changed as needed to maintain dissolved oxygen levels between 8 and 11 ppm and to maintain water temperatures within 2 °C of ambient hatchery tank water. A water conditioner and mucous protectant (Vidalife; Syndel Laboratories Ltd.) was used in all water baths.

Animal care protocols were reviewed and approved annually by institutional animal care committees prior to all fieldwork. Sockeye smolts used in this study were surgically implanted at least 2 days prior to release to allow time for recovery from the anaesthetic and to monitor fish for signs of mortality, tag shedding, and abnormal swimming behaviour. No mortalities were observed. The release of tagged smolts in 2005 was several weeks later than in other years (Table 1) because an electrical failure at the hatchery resulted in the death of almost all surgically implanted smolts prior to their release. Logistical constraints resulted in the subsequent surgery and release of different fish much later (early June 2005) than originally planned (Kintama Research Corp. 2005).

Hydrophone receiver deployments

Acoustic receivers were installed as single or paired units in the lower Fraser River at several locations downstream of the Sumas River confluence. Locations varied between years, with three lines and six receivers in 2004, two lines and four receivers in 2005, three lines (six sublines) and 12 receivers in 2006, and four lines (seven sublines) and 14 receivers in 2007 (Fig. 1).

Ocean detections were recorded in the northern Strait of Georgia (NSOG), Queen Charlotte Strait (QCS), and the Strait of Juan de Fuca (JDF) (Fig. 1). Two acoustic subarrays were also deployed in Howe Sound (HS), a coastal fjord 30 km north of the Fraser River mouth. In 2004 and 2005, Vemco VR2 receivers were used to construct all listening lines using receiver geometry calculated to provide a high probability of detection of any passing tagged smolts. These units were deployed in the spring and recovered in the fall at least 110 days after Cultus Lake sockeye smolts were released, well after the time that smolts were detected crossing ocean lines of receivers. In 2006, VR2 units on NSOG, QCS, and JDF lines initially deployed in the spring were replaced with permanent, year-round VR3 receiver lines during summer 2006. They were uploaded twice in 2007, once in the spring and once in the fall after outmigration. The NSOG and QCS lines remained relatively intact over the course of the study, with <10% loss of receivers per year, even when the more fragile VR2 deployment methodology was used. However, the JDF line was subjected to extensive shrimp bottom trawling and subsequent gear loss in 2004, with one-third of receivers not recovered. In other years, receiver loss was <10% on the JDF line.

Freshwater mobile tracking

To assess the potential for smolts to residualize in freshwater rather than migrate out to sea, we surveyed the number and location of smolts tagged with V9-2L or V9-6L tags in Cultus Lake, the Vedder and Sumas rivers, and Sweltzer Creek for 2 days in late July 2005 (Kintama Research Corp. 2005). We conducted two similar additional surveys in August 2007 to detect any "sleeper" tags (from residualized or dead fish) that were released in 2005 and turned back on in 2007 (Kintama Research Corp. 2008). The 2005 census would not have detected any of the V9-1L sleeper tags released that year as the survey was conducted 4 days after the tags would have turned off. Similarly, the 2007 survey would not have detected any tags released in 2007 as these surveys occurred during programmed sleep periods of tags.

On the first survey day of each year, we used a VR-2 deployed for 6–10 min at 31 fixed stations spaced approximately 500 m apart, covering approximately 95% of the Cultus Lake area. Two days later in 2005, a Vemco VR-28 mobile receiver was used to listen for tags while conducting sampling transects covering approximately 60% of the lake area. In 2007, 1 week after the first survey, we resampled the 31 fixed stations with VR-2 and VR-60 mobile receivers.

Additionally, a network of eight VR-2 receivers was deployed between October 2006 and October 2007 in Cultus Lake for a separate, unrelated study (C. Tovey, Fisheries and Oceans Canada, Pacific Biological Station, Hammond Bay Road, Nanaimo, British Columbia, unpublished data). These receivers detected several 2005 tags that had likely never left the lake and began transmitting again about 30 June 2007 following their sleep period and also several 2007 tags from fish that had residualized, died, or extruded tags in the lake (see Results).

To look for possible mortality locations in the first segment of the migration downstream of Sweltzer Creek (before the stationary Fraser River array), we spent 1 day in 2005 and 2 days in 2007 sampling in the rivers that connect Cultus Lake with the Fraser River. We walked along a 6 km stretch of the Vedder River downstream of the confluence with Sweltzer Creek and lowered a VR-2 receiver into the river at 19 stations, each separated by 200–500 m. In 2007, 600 m of Sweltzer Creek from the Vedder confluence upstream was also sampled. In the lower Vedder Canal, Sumas River, and approximately 2 km of the Fraser River downstream of the Sumas confluence, deeper water permitted tracking by boat. In 2005, we towed a VR-2 receiver and drifted or trolled across these areas, with a single pass being sufficient in the narrow Vedder and Sumas rivers and making three transects in the wider Fraser River section. In 2007, 15 fixed stations in the Vedder Canal and Sumas River were each sampled twice, 1 week apart.

Data analysis

We compiled a database of detections from the stationary receivers consisting of the dates, times, and geographical locations of the detection of each tag. First, we identified and excluded any detections likely to be false (owing to aliasing or tag collisions). Detections were excluded as false if they were detected only once on a line within a 30-min interval, had one or more other tags heard on the same receiver around the time of the suspect detection, and did not have supporting detections from other time periods or lines. Supporting detections are defined as a temporally consistent sequence of detections from release along the migration path. This screening excluded a small number of sporadic detections (0.03%), and the vast majority of the retained data consisted of multiple detections closely spaced in time on a given receiver or subarray. After eliminating suspect detections, we used these filtered data to estimate survival and detection probabilities, migration routes, and travel times and rates of tagged smolts.

Estimation of survival and detection probabilities are described in the next section (see below). To identify possible migratory pathways along the shelf or within the channel, we plotted the number of fish that were heard at each position on the line of acoustic receivers. Because individual fish are usually heard at more than one position on a line, we allocated a proportion of each fish to each of the receivers on which it was detected (i.e., if a fish was heard at three positions, each unit was allocated 0.33 of a fish). We used a χ^2 goodness-of-fit analysis to test if fish were distributed uniformly across the major ocean lines (i.e., each receiver as a separate category). We then grouped fish detected on adjacent receivers to identify broader migratory routes and increase the power of the test. Because the NSOG line is divided by an island, we grouped the fish heard on this line into east or west (i.e., two categories). The QCS line is not divided by an island, so to avoid arbitrarily defining east and west, we ran a series of χ^2 tests in which we grouped neighbouring receivers over an increasingly larger spatial extent (i.e., fewer categories in each test) up to a maximum of five receivers per group (representing potential migration routes ~4 km wide). When the number of receivers per group did not divide evenly, we dropped the receivers on the ends preferentially. We filled in gaps from lost receivers using arithmetic progression from neighbouring units. To test if individuals maintained a preferred migratory route between the NSOG line and the QCS line (240 km to the north), we ran a correlation analysis using the average distance from the mainland that each smolt was detected on each line. Travel times in each segment were measured as the difference between average travel times from release until the first detection of a tag on a line, for successive lines. Travel times from release to the mouth of the Fraser River and from the river mouth to the ocean lines were estimated using the furthest downstream line in the Fraser River each year. The segment distances used in travel rate estimation were measured with mapping software as shortest-route distances between lines of receivers, excluding landmasses.

Survival rate estimation

We used the fully time varying Cormack–Jolly–Seber model (CJS) for live recaptures and variants thereof to simultaneously estimate survival (φ) and detection (p) probabilities in each segment of the migration. To do so, we first determined the detection history of individual fish at each receiver (i.e., recapture) line along the migration path. Sockeye smolts were detected on two to four detection lines in the Fraser River (depending on year) and then on two lines in the ocean (NSOG and QCS) for a total of five, six, or seven detection sites. Only two fish in 2005 and six fish in 2007 were detected on the JDF line, but these were pooled with the QCS detections for the final digit of the CJS tag capture histories to represent exit from the Strait of Georgia system.

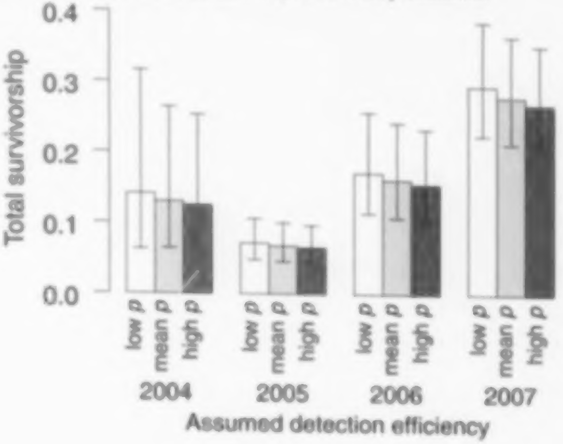
The final survival probability is confounded with detection probability at the final line (QCS–JDF), essentially because there is no more distant location at which the ratio of detected to undetected tags can be assessed. To isolate these factors, we estimated an overall detection probability for the NSOG line (p_{NSOG}) across all four years of the study and assumed this value for the QCS and JDF lines in all years. This approach is reasonable because all three lines had similar receiver geometry during the study period. We used a shorter, three-digit capture history involving release, NSOG recapture, and QCS recapture to more accurately estimate the mean and variance of p_{NSOG}. This estimate of p is the fraction of smolts detected on the QCS line that were also detected on NSOG. Our estimate of p_{NSOG} was for V9 tags from all populations of salmon smolts tagged under POST in 2004–2007 that crossed this line, not just Cultus Lake sockeye. This pooling was done because sample sizes of some populations crossing ocean lines were small and thus population-specific p estimates were highly variable. No consistent seasonality pattern was observed in population-specific p_{NSOG} estimates when compared with their average run timing across this line, so pooling was justified. Further, model selection criteria showed 99.9% support, as measured by Akaike weights, for a model in which species, populations, and years were pooled in a set of four other models involving less pooling of species-, population-, and year-specific estimates of p_{NSOG}. With years pooled, a single estimate of p_{NSOG} was generated and then applied to QCS and JDF as a fixed value in each year. To avoid underestimating uncertainty in the survival estimate for the final migration segment due to fixing this value, survival rates were also calculated by fixing the detection probabilities of QCS and JDF at the lower and upper 95% confidence limits of p_{NSOG}; this bounded a range of uncertainty for the final stage survival estimate and thus the total migration survivorship estimates (Fig. 2).

After assuming fixed values for the final stage detection probabilities, we used the full capture histories in a CJS live recaptures-only model implemented with program MARK (White and Burnham 1999) through RMark (Laake and Rexstad 2007) to estimate φ in river and ocean segments, as well as p on river lines in each year.

Our approach involved two main steps. (*i*) To determine whether survival was best described, on average, as a function of body size, migration distance, and (or) average travel time, we tested several candidate models. We first combined all 4 years in a capture-history data set and assigned "year" as a group covariate. Combining years allowed us to constrain the slope of survival vs. one such covariate to be consistent among years (while permitting intercepts to differ). For example, we constrained the relative survival difference of a big smolt and a small smolt to be equal among years in logit space, even though particular body sizes or overall survival at a particular body size could differ among years.

We considered eight candidate models (Table 2) using this combined data set. Detection probabilities in all models were line-specific and year-specific, $p_{(\mathrm{time} \times \mathrm{year})}$, where the "time" factor represents recapture locations at receiver lines. One survival model was the classic time-varying CJS model, $\varphi_{(\mathrm{time} \times \mathrm{group})}$, with separate segment-specific survival estimates for each of the 4 years. Another model incorporated the additive effect of body size as an individual covariate into the CJS model. The other six models involved minimum migration distance or average travel time group covariates to replace the freely varying segment-specific estimates. These constrained models, some of which included body size as a covariate, involved fewer parameters. Some allowed for separate effects of in-river and ocean segments of distance or travel time on survival, and others were further constrained without this multiplicative habitat effect. We estimated a variance inflation factor ($\hat{c}$) to compensate for overdispersion, or extra-binomial variation, in estimated probabilities (Burnham et al. 1987). We estimated $\hat{c}$ (= 1.3325) using the deviance ratio method in MARK's goodness-of-fit bootstrapping routine assuming the general CJS model, $\varphi_{(\mathrm{time} \times \mathrm{year})}p_{(\mathrm{time} \times \mathrm{year})}$. QAIC$_c$ values, corrected for extra-binomial variation and sample sizes, were computed for model comparison.

Fig. 2. Survivorship estimates from release to exit from the Strait of Georgia system for different assumed detection probabilities on the final Queen Charlotte Strait (QCS) and Strait of Juan de Fuca (JDF) lines. The mean and lower and upper 95% confidence limits of northern Strait of Georgia (NSOG) detection probabilities, p, are assumed for QCS and JDF detection probabilities. Error bars show 95% confidence limits on resulting survivorship estimates.



For step *ii*, once we determined the best-supported models on average from the eight models described above, we analyzed the 4 years independently so that we could estimate the extra-binomial variation, $\hat{c}$, for each year of the study. By estimating $\hat{c}$ annually, the variance around each of the estimated probabilities is specific to the variation observed within that year (and not all years combined). Using the bootstrapping routine again, we estimated $\hat{c}$ for the fully time varying CJS models as 1.73, 0.59, 1.44, and 1.28 in 2004–2007, respectively. The $\hat{c}$ value estimated at <1 in 2005 was set to 1 (Cooch and White 2007). These $\hat{c}$ values were used to expand standard errors of real parameter estimates and values in the variance–covariance matrix.

The top two candidate models had almost equal support and together had nearly 100% of the support within the set of eight models considered, and therefore we considered only these two models for each individual year. We computed model-averaged parameter estimates for φ and p at each segment or detection station for that year. For each sequential receiver station, we calculated survivorship from release to that point as the product of segment-specific φ estimates. We used the delta method to calculate the variance of this product.

Table 2. Model selection results for recaptures-only survival probability estimates.[a]

Model	No. of parameters	$QAIC_c$	$\Delta QAIC_c$	Akaike weight
$\varphi(\text{time} \times \text{year+body})\,p(\text{time} \times \text{year})$[b]	43	2284.5	0.0	0.52
$\varphi(\text{time} \times \text{year})\,p(\text{time} \times \text{year})$	42	2284.6	0.1	0.48
$\varphi(\text{TravTime+habitat} \times \text{year+body})\,p(\text{time} \times \text{year})$[b,c]	28	2309.1	24.6	0.00
$\varphi(\text{TravTime+habitat} \times \text{year})\,p(\text{time} \times \text{year})$	29	2309.3	24.8	0.00
$\varphi(\text{distance+habitat} \times \text{year+body})\,p(\text{time} \times \text{year})$	28	2394.2	109.8	0.00
$\varphi(\text{distance+habitat} \times \text{year})\,p(\text{time} \times \text{year})$	29	2394.2	109.8	0.00
$\varphi(\text{TravTime+year})\,p(\text{time} \times \text{year})$	24	2441.7	157.2	0.00
$\varphi(\text{distance+year})\,p(\text{time} \times \text{year})$	24	2465.1	180.6	0.00

[a] AIC values are adjusted for small sample sizes and extra-binomial variation with $\hat{c} = 1.3325$.

[b] Time in models refers to segment- or line-specific probabilities, whereas TravTime is a group covariate and refers to average travel time of the population in a particular segment of the migration.

[c] Habitat in models means that the intercept between survival and either distance or travel time was allowed to differ in river and ocean segments. Models with an interaction term added so that the slope was also allowed to differ (TravTime × habitat and distance × habitat) were also tested, but these reduced AIC by less than 2, so the incorporation of the extra parameter was not supported.

Fig. 3. Survivorship relative to cumulative distance migrated for acoustically tagged Cultus Lake sockeye. Vertical bars represent 95% confidence intervals; 2004, ■; 2005, ◆; 2006, ▲; 2007, ▼. NSOG, northern Strait of Georgia; QCS, Queen Charlotte Strait.

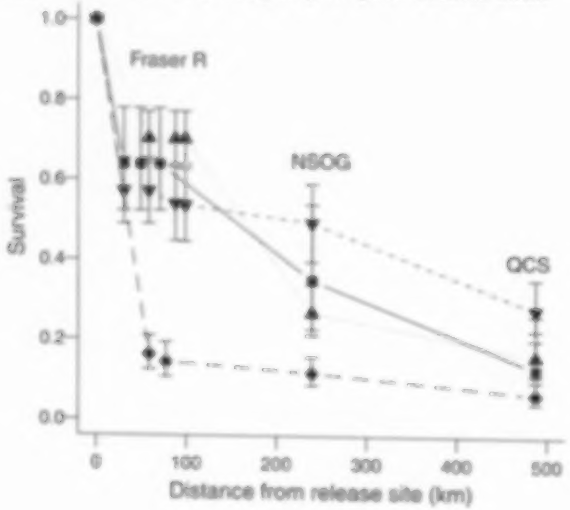
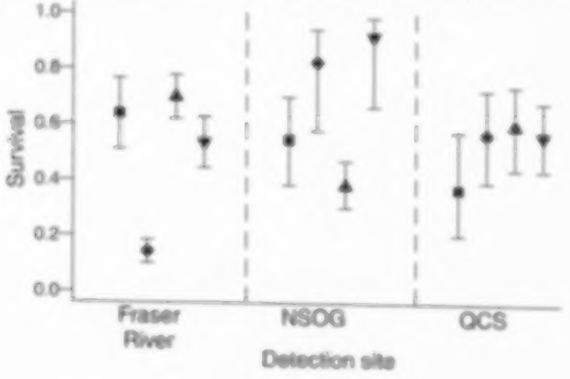


Fig. 4. Segment-specific survival estimates for acoustically tagged Cultus Lake sockeye from release to the lower Fraser River, lower Fraser River to northern Strait of Georgia (NSOG), and NSOG to Queen Charlotte Strait (QCS), 2004–2007. Vertical bars represent 95% confidence intervals; 2004, ■; 2005, ◆; 2006, ▲; 2007, ▼.



Results

Survival estimates

Model selection

Of the eight survival models evaluated across all years, the strongest support was found for two models: the fully time varying CJS model (with year- and segment-specific estimates); and the fully time varying CJS model that incorporated body size as an individual covariate (Table 2). These two models had essentially equal support, as seen in their Akaike weights. These models differed by a single parameter, so the small difference in AIC suggests little support for the larger model overall. However, when analyzed separately, 2004 and 2006 had somewhat more support for the model incorporating body size ($\Delta QAIC_c > 3$), so these models were both used for averaging the year-specific estimates. The six models with migration distance or average travel time constraints had fewer parameters but had much poorer fits (as measured by the overall likelihood of each model), so had little support from the data within the model set.

Survival probability estimates

With each year analyzed separately, the model-averaged estimates of the top two models (Table 2) show decreasing survivorship over the first 500 km of the juvenile salmon migration (Fig. 3). The "low p" (or "high p") estimates reported show the estimated survivorship to exit from the Strait of Georgia system assuming that the value of p_{QCS} is set to the lower (or upper) 95% confidence interval of p_{NSOG} rather than the mean estimate (Fig. 2; see Materials and methods). Because this bounded range of survivorship estimates is relatively small (compared with yearly differences, for example), and because much mortality occurred before the final segment, our survivorship estimates appear to be fairly robust to uncertainty in p_{QCS}.

The 2005 cohort stands out with markedly lower mean

Table 3. Migratory behaviour categories for Cultus Lake sockeye in 2004 to 2007.

Migration behavior	2004	2005	2006	2007
North (direct)	21(21.0)	37 (9.8)	40 (20)	69 (34.5)
North through HS	9 (9.0)	6 (1.6)	13 (6.5)	8 (4.0)
South	0	2 (0.5)	0	6 (3.0)
Milling on NSOG	0	5 (1.3)	5 (2.5)	5 (2.5)
Milling on QCS				1 (0.5)
Milling on JDF		1 (0.27)		1 (0.5)
Milling on Fraser	0	0	10 (5.0)*	1 (0.5)
Residualization in Cultus Lake	Not surveyed	17 (4.5)	Not surveyed	12 (6.0)

Note: The first number in each column is the number of fish detected. The number in parentheses indicates the percentage of fish detected in relation to all fish released that year. Milling is defined as being detected either for >24 h on a line or (on Fraser River lines) downstream, upstream, and then downstream again. HS, Howe Sound; NSOG, northern Strait of Georgia; QCS, Queen Charlotte Strait; JDF, Strait of Juan de Fuca.

*Five of these 10 fish were detected downstream, upstream, then downstream again. Three of these five were subsequently heard on ocean lines and two were not; we suspect that these two tags may have been in seals. The remaining five fish were heard on a Fraser River line for >24 h (3 fish) or moved between subarrays within stations F or G (Fig. 1), thereby requiring upstream travel (2 fish).

Table 4. Residualization and initial downstream migration surveys of Cultus Lake and the rivers that connect it to the Fraser River.

Detection year	Tag year	Cultus Lake	Sweltzer Creek	Vedder River	Lower Vedder Canal	Lower Sumas River	Fraser River
Mobile surveys							
2005	2005	13	NA	0	0	2	0
2007	2005	6	0	0	0	0	NA
	2007	NA	NA	NA	NA	NA	NA
Number of 2005 tags heard in both years							
	NA	5	NA	0	0	0	NA
Additional tags heard in DFO survey (not heard in either mobile survey)							
2007	2005	7	NA	NA	NA	NA	NA
	2007	12	NA	NA	NA	NA	NA

Note: NA, not applicable. Sweltzer Creek was not surveyed in 2005, and the Fraser River was not surveyed in 2007.

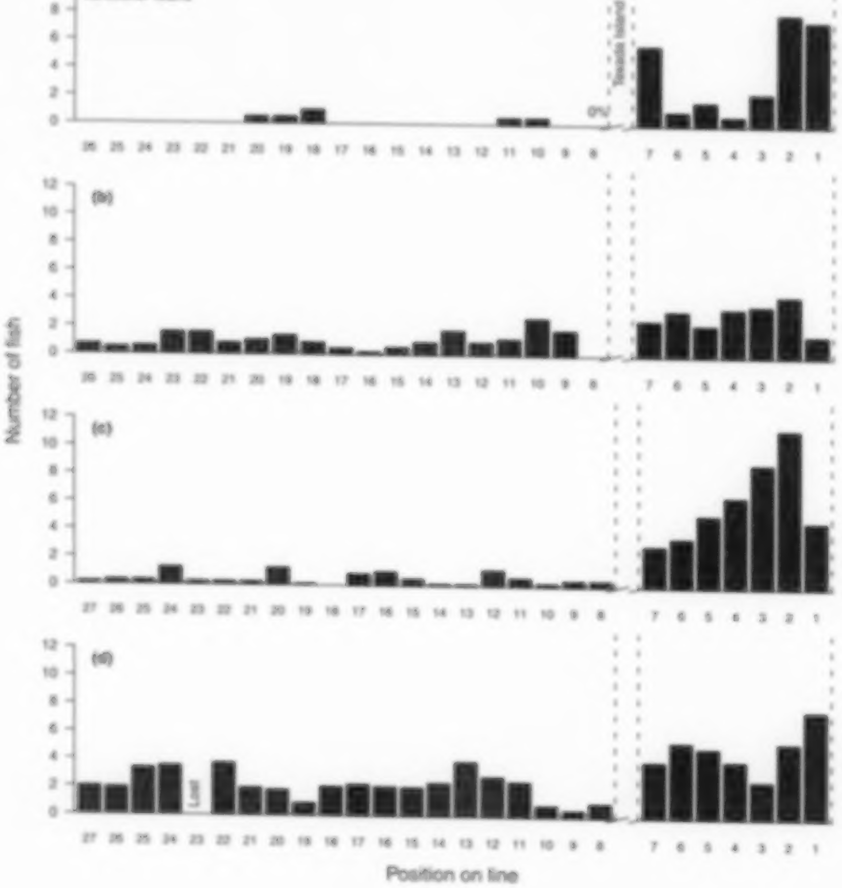
survival (0.12) compared with the other years, especially in the freshwater component of the migration where survival was less than one-third that of the other years (0.5–0.7). Taken across all years, mean survivorship to the northern Strait of Georgia ranged from 0.1 to 0.5, whereas survivorship to the final line in Queen Charlotte Strait ranged from 0.07 to 0.28 (Fig. 3).

Survivorship to the outer lines was probably underestimated in 2006 and possibly in 2007. Smolt tags in 2006 were programmed to shut off between 1 and 4 June (and to begin retransmitting 2 years later during the adult return migration), but some surviving outbound smolts may not have crossed the QCS line before the tag's transmissions were stopped. The mean date of first crossing the QCS line was 25 May (0216 hours), but a few fish ($n = 3$) were still detected on 1–2 June. The likelihood that a few fish crossed the line after their tags were turned off is also supported by the date of crossing the NSOG line: the mean crossing date was 8 May (1052 hours) for fish that were detected on QCS ($n = 30$), and 10 May (2311 hours) for fish not detected on QCS ($n = 24$). Individuals late in crossing the NSOG line thus also tended to be the individuals not detected on QCS. Similarly, tags in 2007 were programmed to shut off around 27–28 June, and a few fish were still detected on the QCS line between 20 and 25 June. Thus, some slower-moving smolts could have crossed the QCS line undetected; four of the five tags last detected on the NSOG line (between 10 and 17 June) were not detected on the QCS line. However, the potential effect of a premature shutdown of active transmission is likely to have little effect on the overall estimate of survival to QCS, as the majority of detected animals reached the QCS line at least 2 days prior to tag shutdown (27 of 30 detected survivors in 2006; 43 of 44 detected survivors in 2007).

Segment-specific survival estimates (Fig. 4) provide a way to directly assess Cultus Lake smolt survival in various segments of their migration path. The markedly lower freshwater survival in 2005 stands out. However, the 2005 cohort had relatively early high marine survival, indicating that the reduced survival observed in freshwater did not persist in the ocean. The Fraser–NSOG segment survival for 2006 indicated significantly lower marine survival (despite high freshwater survival that year) compared with both the 2005 and 2007 cohorts. There were no marked trends in marine survival within the NSOG–QCS segment other than the slightly lower (and more uncertain) value measured in 2004, and there was considerable overlap in all 95% confidence intervals.

Our reported survival estimates depend on the simultaneously estimated detection probabilities at the various subarrays (or listening lines) that we deployed. Detection probabilities on individual receiver lines in the Fraser River

Fig. 5. Distribution of migrating acoustically tagged Cultus Lake sockeye on the northern Strait of Georgia lines: (*a*) 2004; (*b*) 2005; (*c*) 2006; (*d*) 2007. Each numbered bar represents a receiver in the line. If a fish was heard on multiple receivers, that individual's detection was allocated equally across all receivers on which it was detected. The 0% above receiver 8 in 2004 indicates that this receiver was not operational during the time when the fish were crossing the line. Receiver 23 in 2007 was not recovered.



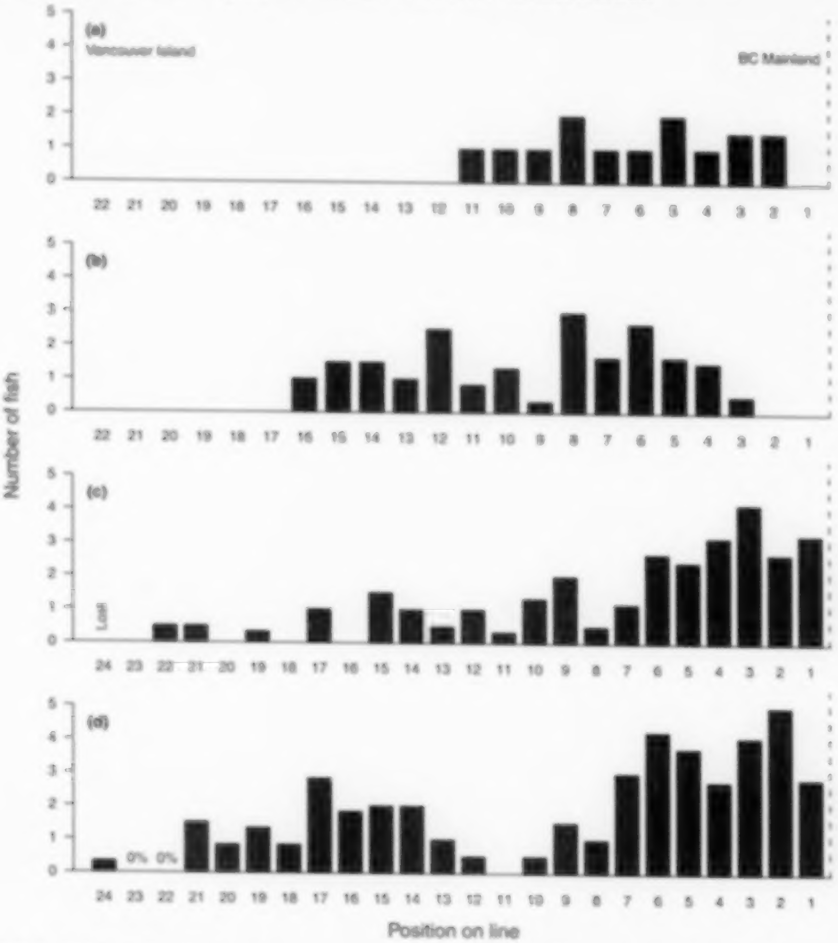
(p_i) ranged from 14% to 98% across all years (average of 64%). Taking the product of $(1 - p_i)$ for all Fraser River lines in each year results in the probability of a smolt crossing all Fraser lines without being detected (Melnychuk et al. 2007). This value (2004, 0.7%; 2005, 23%; 2006, 0.03%; 2007, 7.8%) is the nondetection rate for the Fraser River subarray as a whole; thus, in most years, aggregated detection probabilities for the lower Fraser River subarray exceeded 90%. Detection probabilities on the NSOG line averaged 90% (range 85%–93%). As discussed earlier, this value was assumed for the QCS–JDF lines, as the geometry of these subarrays is very similar.

Migration behaviour

Cultus Lake sockeye displayed four predominant migratory behaviours (Table 3). Northward migration in the ocean was the most common behaviour observed, which accounted for 9.8%–34.5% of all released fish each year. These fish migrated quickly out of the Fraser River and then had a directed migration crossing the NSOG and QCS lines. Migration north but first entering Howe Sound prior to heading across the two northern lines of NSOG and QCS occurred in 1.6%–9% of all released fish each year. Only eight fish, two (0.5% of released fish) in 2005 and six (3% of released fish) in 2007, were ever detected swimming south from the Fraser River (Table 3). However, it should be noted that one-third of the receivers on the JDF line were lost in 2004, so it is possible that some fish migrated south through these gaps and were not detected in 2004. Two of the six fish crossing the JDF line in 2007 were also detected later on the Lippy Point line (Fig. 1). There was some evidence of fish milling within the Fraser River and in the vicinity of ocean lines (Table 3).

Some tagged fish moved upstream after release in Sweltzer Creek into Cultus Lake (Tables 3 and 4) during both of the years in which freshwater surveys were conducted (4.5%

Fig. 6. Distribution of migrating acoustically tagged Cultus Lake sockeye on the Queen Charlotte Strait line: (*a*) 2004; (*b*) 2005; (*c*) 2006; (*d*) 2007. Each numbered bar represents a receiver in the line. If a fish was detected on multiple receivers, that detection was allocated across all receivers on which it was detected. The 0% above receivers 22 and 23 in 2007 indicates that these receivers were not operational during the time when the fish were crossing the line. Receiver 24 in 2006 was not recovered.



in 2005 and 6.0% in 2007). After this initial upstream movement, fish could have remained within Cultus Lake without migrating out of freshwater, died in the lake, or shed their tags in the lake. Four of the 12 fish detected in the 2007 surveys were detected at the same stations as in 2005, which suggests that these were probably tags lying on the lake bottom. It is unclear whether the tags only detected on a single survey were present in moving (and therefore surviving) sockeye, but this seems likely for at least some tags.

Distribution across receiver curtains

Because POST lines in the Strait of Georgia are bordered by land on both sides and because detection probabilities were high in all years, it was possible to explore the distribution of migrating smolts at sea and establish whether fish had preferred "migration highways". Smolts crossing the NSOG line (Fig. 5) showed a marked preference for the mainland route east of Texada Island in 2004 and 2006 ($p < 0.001$) when compared with a uniform distribution across all receivers on the line (i.e., each receiver treated as a separate category in the χ^2 analysis). This preference was not evident in 2005 and 2007 ($p > 0.37$). When fish were grouped according to whether they migrated on the east versus the west side of Texada Island (two categories; Texada Island splits the NSOG line into two sections), they showed a significant preference for the eastern channel (all years analyzed separately, $p \leq 0.001$).

The distribution of smolts crossing the QCS line (Fig. 6) was not significantly different from uniform when each receiver was treated as a separate category in the χ^2 analysis (2005–2007 years tested separately, $p > 0.24$; 2004 insufficient data). However, when the detections on neighbouring receivers were aggregated together (i.e., extending the spatial area and reducing the number of categories), the χ^2 tests became increasingly significant as the area of aggregation increased (Fig. 7). Fish showed significant preference for the eastern side of the Strait in 2004, 2006, and 2007 ($p = 0.04$, $p < 0.001$, $p = 0.002$, respectively, over a 3.2 km extent) and near-significant selection for the eastern-central channel in 2005 ($p = 0.11$).

A correlation analysis showed no relationship between where individuals crossed the NSOG line (estimated as the average distance from the B.C. mainland) and where they crossed the QCS line 240 km to the north ($r^2 = 0.04$–0.2; 2004–2007).

Travel rate and swimming speeds

Cultus Lake smolts exited the Fraser River in <6 days. The later-released fish in 2005 and 2007 travelled faster, with mean times to the river mouth of 4.1 and 4.0 days, respectively. The earlier-released fish in 2004 and 2006 took slightly longer to reach the river mouth (5.6 and 5.0 days, respectively). There was no clear pattern with release date and time taken to exit the Strait of Georgia across the QCS line. Average times from release to reaching the QCS line ranged from 25.6 to 34.1 days.

Travel rate was variable among years and for different sections of the migration route, with mean values ranging between 5 and 30 km·day^{-1} (Fig. 8; 0.46–1.8 body lengths (BL)·s^{-1}). Fish tagged in 2004 had the slowest migration rate in the Fraser River, with a mean speed of 15.5 km·day^{-1}. However, these fish had the fastest mean swimming rate in the marine sections when swimming directly to both the NSOG and QCS lines. The 2007 cohort had the fastest rate of movement out of the Fraser River (25.8 km·day^{-1}) but relatively slow rates in the ocean segment from the river mouth to either HS or NSOG. In most years, travel rates in the NSOG to QSC segment were faster than in other segments, around 26 km·day^{-1}.

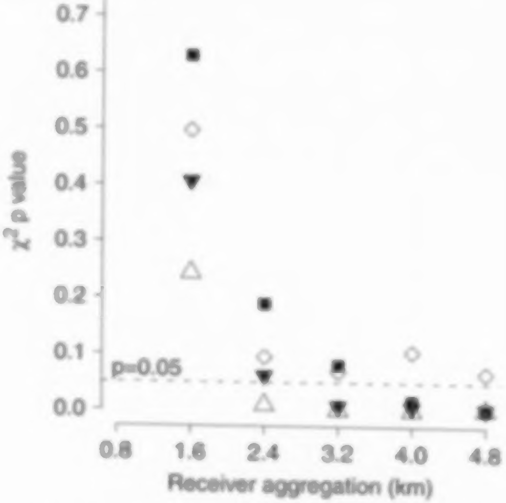
Discussion

Survival

A significant constraint on salmon conservation and management is our poor understanding of mortality during different life-history stages after leaving freshwater. Poor marine survival has been implicated for the drastic decline of many salmon (McKinnell et al. 2001; Beamish et al. 2004; English et al. 2008) and steelhead (Ward 2000; Welch et al. 2000) stocks in southern B.C. This study presents a first step in monitoring both freshwater and early marine survival of endangered Cultus Lake sockeye during the initial stages of migration.

Survival rates during the freshwater migration phase were relatively stable among the years studied, at 50%–70% of migrating smolts (except 2005, when survival was <20%, possibly because of the very late release). However, given the relatively short distances that must be travelled to the mouth of the Fraser River (≤100 km), the short duration of the freshwater migration, and the absence of obvious impediments to travel such as dams, the specific survival levels observed are surprisingly low. Our results do seem consistent with freshwater survival estimates for other west coast salmon populations: Keogh River steelhead, 75% (Welch et al. 2004); Cheakamus River steelhead, 80% (Melnychuk et al. 2007); Thompson River steelhead, 30%; Thompson River Chinook, 23%; Snake River steelhead, 18%; and Snake River Chinook, 25% (Welch et al. 2008). The higher survival estimates are seen for smolts travelling much shorter distances to the sea (ca. 0.3–16 km), and the lower survival estimates are for smolts travelling much farther to the sea (ca. 300–900 km); the Cultus sockeye results generally fall in the middle of this range.

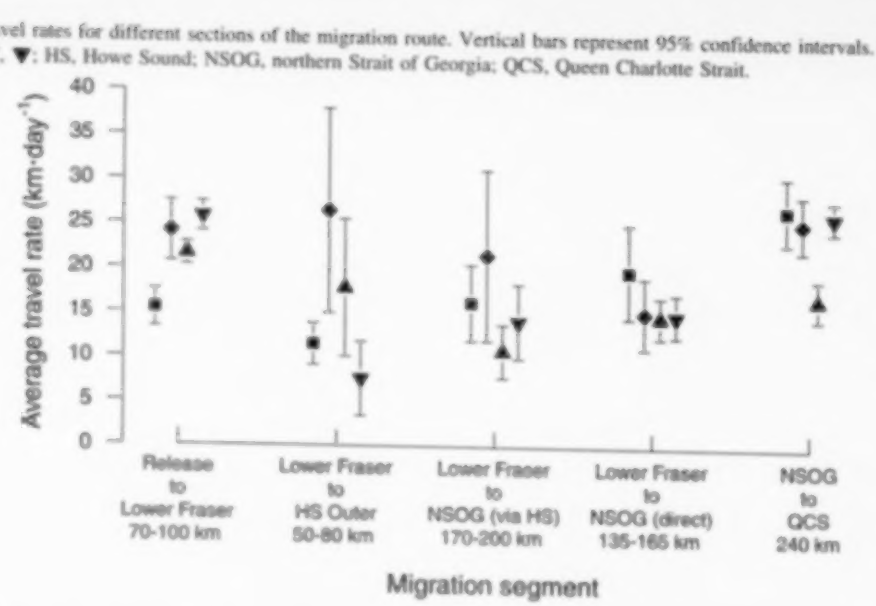
Fig. 7. Significance of a series of χ^2 goodness-of-fit tests obtained by grouping fish detected on neighbouring receivers. For spatial aggregations > 3 km (i.e., aggregating the data from four or more adjacent receivers), statistical support for significant spatial pattern on the Queen Charlotte Strait subarray is evident. 2004, ■; 2005, ◇; 2006, △; 2007, ▼.



Several factors may have caused the high freshwater mortality observed in 2005. First, these hatchery-reared fish were released much later than in other years because a power failure killed almost all of the smolts that were originally implanted (Kintama Research Corp. 2005). Later release times have been associated with poorer survival in other studies (Bilton et al. 1982), and the lower water levels in Sweltzer Creek during June compared with May might make the fish more susceptible to predators. (Three smolts were observed being taken immediately by birds after release that year.) Second, a fence normally sited in Sweltzer Creek to reduce the frequency of fish migrating back up into Cultus Lake was removed on 1 June 2005, which meant that some smolts could have migrated back upstream (however, the lake survey in 2005 revealed similar levels of residualization as in 2007 when the fence was in place).

Marine survival rates were also stable across years (including 2005) and were consistent with survival estimates for the nearby Cheakamus River steelhead (Melnychuk et al. 2007). The 2005 sockeye smolt outmigration year resulted in exceptionally poor adult returns coastwide in 2007 (Fisheries and Oceans Canada (DFO) 2008); however, our results for 2005 do not show a substantial difference between years in early ocean survival in the Strait of Georgia (SOG), which is consistent with independent information provided by a DFO SOG trawl survey for juvenile salmon (DFO 2008, p. 13). This could be explained by several possibilities. First, the SOG may have been insulated from events occurring more broadly on the west coast and thus higher mortality at sea could have occurred later, beyond

Fig. 8. Average travel rates for different sections of the migration route. Vertical bars represent 95% confidence intervals. 2004, ■; 2005, ◆; 2006, ▲; 2007, ▼; HS, Howe Sound; NSOG, northern Strait of Georgia; QCS, Queen Charlotte Strait.



the SOG. Second, the low abundances of salmon smolts in the ocean in these other regions could also have been the result of poor downstream survival in other natal rivers. Third, the relatively high survival of Cultus Lake sockeye smolts through the Strait of Georgia system in 2005 may have been the result of the fittest individuals surviving the high freshwater mortality phase and thus migrating through the Strait of Georgia with relatively high survival.

Overall, survivals showed a relatively steady attrition with distance and do not indicate particular areas of higher mortality at the spatial resolution of this study. Unfortunately, we have no comparable data on baseline survival rates for the earlier period of much better adult returns that occurred prior to the deployment of POST in 2004.

It should be noted that the survival estimates presented here using the pilot phase of the POST array might not accurately represent survival in the overall Cultus Lake sockeye population. We sampled smolts that were about 50% longer than wild free-ranging smolts to accommodate the V9 acoustic tags that the "first generation" POST array was originally designed around in 2003. As salmon survival scales positively with size when considered over a wide range of sizes (McGurk 1996, 1999), we would expect that the survival of the wild (smaller) smolts to be potentially lower than what we measured here.

The survival estimates presented here may also be biased as a result of two confounding factors. First, total survival estimates reported for 2005 and 2007 are biased high as a result of overestimating survival in the segment from the river mouth to NSOG, due to the confounding factor of small numbers of fish swimming south after ocean entry in those years. However, as noted earlier, this bias is expected to be minor as only two fish were detected migrating south through Juan de Fuca in 2005 and only six fish were detected in 2007. Second, survival is actually a joint estimate of the probability of migration and survival. Residualization (nonmigration) in freshwater (either Cultus Lake or the streams and rivers leading to the Fraser River mouth) is confounded with mortality during the downstream migration, so our reported survival estimates are biased low to some degree. However, the surveys carried out in 2005 and 2007 suggested a relatively low minimum residualization rate of between 5% and 6% of all tagged fish.

Migration highways

The POST lines (particularly those between the mainland and Vancouver Island) provide a unique means to identify whether salmon have preferred migration routes or "highways". Limited evidence suggests that specific "marine highways" may be as common under the ocean (e.g., Atlantic cod off Newfoundland; Rose 1993) as they are observed to be on land for mammals and birds. Our results for Cultus Lake sockeye confirm the importance of the Johnstone Strait migration pathway proposed for migrating juvenile Fraser River sockeye by Groot and Cooke (1987). These authors found that smolts predominantly move north in a directed migration with a preference for staying to the east side of the Strait of Georgia and Johnson Strait. Our results further extend the persistence of this migratory preference to Queen Charlotte Strait, some 500 km from the release site. As in our study, Groot and Cooke (1987) also found that Fraser River sockeye enter Howe Sound. The ecological reason for these migration routes in some years is unclear, as are the reasons for the interannual variation in saltwater distribution that we have documented with the array.

Cultus Lake sockeye displayed relatively rapid, directed movement once they commenced their downstream migration with little evidence of milling. Earlier work on sockeye salmon calculated average freshwater migration speeds of between 5.1 and 7.8 km·day^{-1} in lakes but up to 40 km·day^{-1} in the Columbia River (Burgner 1991). Our results indicated travel rates of between 15 and 25 km·day^{-1} for Cultus Lake sockeye. Obviously, river flow is an important influence on travel rate, although the results show that sustained travel rates of 10–30 km·day^{-1} were also achieved in the marine inshore environment. Thus continued rapid migration occurred without the influence of river flow. The faster downstream migration observed in 2005 and 2007 is likely related to release time, as river flow generally increases later in the season, exposing the 2005 and 2007 cohorts to greater stream flows.

The mean swimming speeds of Cultus sockeye smolts of 0.5–1.8 BL·s^{-1} in both freshwater and marine environments is comparable with results from acoustic tracking studies on other salmonids. Freshwater and marine swimming speeds of steelhead trout in southern British Columbia waters were found to be between 0.5 and 2.0 BL·s^{-1} (Welch et al. 2004; Melnychuk et al. 2007). In western Atlantic waters, Thorstad et al. (2007) recorded swimming speeds of 0.53 and 0.56 BL·s^{-1} for wild and hatchery Atlantic postsmolts, respectively (but only 0.06 BL·s^{-1} for brown trout postsmolts) in a Norwegian fjord.

The POST array

Our work represents the first direct estimates of survival and movement rates of sockeye (*Oncorhynchus nerka*) smolts during the freshwater and early marine phases of the migration period and demonstrates the utility of large-scale telemetry arrays for monitoring smolt migrations over repeated years. Acoustic technology, properly implemented, provides an essentially seamless monitoring system covering both freshwater and marine environments. It is thus possible to study salmon smolt migrations along the full length of their migration along the continental shelf as they move out of their natal stream. The POST array provides a level of resolution not previously available in marine studies and allows us to specify the speed, directionality, migration routes, and survival of tagged smolts with substantial precision even when using modest numbers of tagged smolts. Pinpointing areas of potentially elevated levels of mortality is of particular relevance to endangered stocks of salmon, as this can help direct research towards the most relevant parts of the life history.

The results from this study demonstrate that it is technically feasible to measure survival in both large river and coastal marine environments. Further miniaturization of the tags now allows smolts in the 100 mm TL range to be tagged, but at a substantially increased cost for operating an array of equivalent detection efficiency and thus statistical precision. However, a key point is that technical advances will thus likely allow future array designs to be extended to smaller fish and potentially resolve some of the limitations associated with only tagging larger smolts.

In summary, our study provides useful new data on Cultus Lake sockeye smolt migration and mortality during an important life-history period when the transition occurs from freshwater to marine life. The development of baseline data for salmon stocks can assist with managing them under climate change, not just by monitoring changes in survival, but also by allowing the formal testing of experimental hypotheses about the factors controlling salmon abundance. The POST array concept also provides a useful demonstration of the potential of continent-scale acoustic arrays for simultaneously monitoring a wide range of marine species for conservation and management. Such a concept is now being developed worldwide in the global initiative of the Ocean Tracking Network, which is establishing acoustic lines and arrays at strategic locations around the world (O'Dor et al. 2007). Developments like those reported here will help to incorporate biological observations into broader global ocean-observing systems.

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Verification of Atlantic sea scallop (*Placopecten magellanicus*) shell growth rings by tracking cohorts in fishery closed areas

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Abstract: We tracked the growth of large cohorts of sea scallops (*Placopecten magellanicus*) at four sites located in areas closed to scallop fishing and compared the observed growth with that inferred from rings on sea scallop shells collected at the same sites. Stochastic growth transition matrices were constructed for each site based on the shell growth increments, assuming annual ring formation. These matrices were used to predict the annual growth of the scallops, which were compared with direct observations of growth obtained by repeated sampling. Additionally, the observed growth of the scallops was used to estimate the parameters of a stochastic von Bertalanffy model for each site, which were used to estimate the mean annual growth increments as a function of starting shell height. These were compared with the mean growth increments on the shells. There was a close correspondence, in most cases, between the observed growth and the growth inferred from the shell rings, implying that the shell rings were formed annually. The lack of fishing mortality in the areas meant that there was no confounding of size-selective fishing with growth and allowed us to track cohorts longer than would otherwise have been possible.

Résumé : Nous avons suivi la croissance de grandes cohortes de pétoncles géants (*Placopecten magellanicus*) à quatre stations situées dans des zones interdites à la pêche de pétoncles et comparé la croissance observée à celle déduite de la lecture des anneaux de la coquille de pétoncles géants récoltés dans les mêmes stations. Nous avons élaboré des matrices stochastiques de transition de la croissance pour chaque station d'après les incréments de croissance des coquilles, en présupposant que la formation des anneaux de croissance est annuelle. Ces matrices ont servi à prédire la croissance annuelle des pétoncles, qui a pu être comparée aux observations directes de la croissance obtenues par échantillonnage répété. De plus, la croissance observée des pétoncles a servi à estimer les paramètres d'un modèle stochastique de von Bertalanffy pour chaque station qui ont permis d'estimer les incréments annuels moyens de croissance en fonction de la hauteur initiale de la coquille. Il y a, dans la plupart des cas, une forte correspondance entre la croissance observée et la croissance déduite des anneaux de la coquille, ce qui laisse croire que ces anneaux de la coquille se forment annuellement. L'absence de mortalité due à la pêche dans ces zones fait qu'il n'y a pas de confusion entre la pêche sélective en fonction de la taille et la croissance, ce qui nous a permis de suivre les cohortes pendant plus longtemps qu'il n'aurait été possible autrement.

[Traduit par la Rédaction]

Introduction

Besides their use for conservation and management purposes, fishery closed areas, often called marine protected areas (MPAs), can provide important opportunities for scientific research. Although the primary scientific use of such areas has been as reference areas to infer the effects of fishing, they can be useful for other purposes as well. For example, Willis and Millar (2005) used an MPA to help estimate fishing mortality in the adjacent fished areas. Here we use MPAs to verify the annual nature of growth rings or marks on the shells of the sea scallop, *Placopecten magellanicus*, off the northeastern coast of the United States.

Early work examining monthly shell samples from the Bay of Fundy (Stevenson and Dickie 1954) and comparing growth from shell rings with that inferred from tagging on Georges Bank (Merrill et al. 1966) concluded that sea scallop growth rings are laid down annually. Krantz et al. (1984) used stable isotope analysis to age two shells from the Mid-Atlantic Bight and, based on a comparison of the isotope analysis with the visual shell rings, suggested that two rings are formed per year. A similar comparison of stable isotope analysis with the visual rings from two shells from Brown's Bank was more supportive of the one ring per year hypothesis, although not definitively conclusive (Tan et al. 1988).

The aim of our study was to directly track the growth of large cohorts at selected sites within MPAs by repeated sampling. The lack of fishing within these areas meant that growth could be observed without the confounding effects

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Table 1. Latitude and longitude (decimal degrees), water depth (metres), years in which size frequencies and shell samples were taken, and mean size-frequency sample sizes for each of the four study sites.

Site	Latitude	Longitude	Depth	Size-freqency samples	Shell samples	Mean sample size
1	41.14	66.82	71	2000–2003, 2005	2003–2006	570
2	41.07	66.88	70	2000, 2002, 2004–2006	2004–2007	648
3	40.57	69.71	61	1999–2000, 2003–2008	2003–2008	339
4	38.56	73.80	58	2003–2008	2004–2008	732

of fishery selectivity and removals. This observed growth could then be compared with that inferred by the shell growth rings.

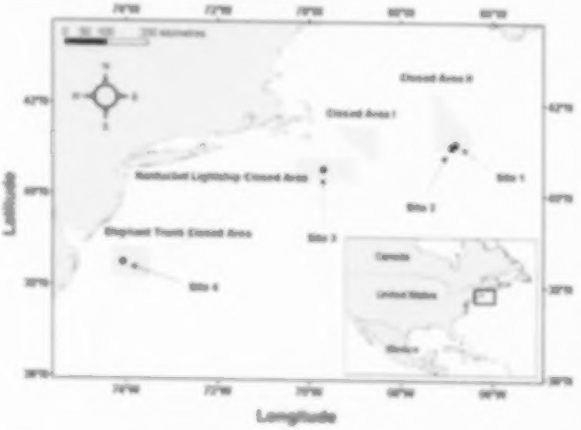
Methods

Four sites, spread over the range of the sea scallop in US waters, were chosen for this study. At each site, large numbers of small scallops were initially observed during random sampling as part of the annual sea scallop survey conducted by the Northeast Fisheries Science Center (NEFSC). These sites were then resampled during subsequent years during the survey so that the growth of scallops could be estimated using the changes in the size frequencies over time. Two of these sites (sites 1 and 2) were located in Georges Bank Closed Area II and were initially sampled in 2000 (Table 1; Fig. 1). Site 3, located in Nantucket Lightship Closed Area, was originally sampled in 1999, and site 4 in the Elephant Trunk Closed Area off Delaware Bay was initially sampled in 2003. The survey was conducted during July and August of each year; the southern areas (including site 4) were sampled in July, and Georges Bank (sites 1 and 2) was sampled in August. All of these sites were located in areas closed to scallop fishing for most or all of the study period (Hart and Rago 2006). At the two Closed Area II sites (sites 1 and 2), only a relatively small number of scallops were observed in 2004 and 2006 (site 1) and 2007 (site 2). These size frequencies were judged to be too unreliable and hence were not used in the analysis.

The scallops were sampled using a modified New Bedford scallop dredge with 5.1 cm rings and a 3.8 cm polyethylene mesh liner. Tows were conducted for 15 min at an average speed of 3.8 kn. With two exceptions (site 3 in 2000 and site 1 in 2003), sampling at each site and year consisted of a single tow. Sea scallops caught in the dredge were measured to the nearest 5 mm shell height on mechanical measuring boards prior to 2005 and to the nearest 1 mm on electronic measuring boards from 2005 onwards. Survey dredges show flat selectivity for scallops of 40 mm shell height or greater (NEFSC 2007); when catches were very high, as was the case in the initial year at all of these sites, the dredge likely nearly maintains this selectivity to even smaller shell height due to clogging of the liner and rings with scallops.

Starting in 2003 (2004 for sites 2 and 4), between 60 and 100 scallops per site were collected each year for shell ring analysis. The scallops were cleaned and shucked, and then both valves were numbered and brought to shore for measurement of growth increments, defined as the distance between visible growth rings on the upper valve. For every shell, the distance from the umbo to each ring was measured, so that the difference between consecutive measurements would represent an annual growth increment. The new shell material deposited after the final ring was not used as it represented only a partial year's growth.

Fig. 1. Map showing locations of the four study sites. Sites 1 and 2 are in Closed Area II on Georges Bank. Site 3 is in the Nantucket Lightship Closed Area on Nantucket Shoals, south of Nantucket Island. Site 4 is located in the Elephant Trunk Closed Area, east of Delaware Bay in the Mid-Atlantic Bight.



We used two methods to compare the predicted growth derived from the shell rings with the growth observed by repeated size-frequency sampling. In the first method, the observed growth rings were grouped into 5 mm shell height bins. For the shells that had a ring in bin j (excluding any where this was the largest ring), let f_{ij} be the fraction of these shells in which the next ring was in bin i. In other words, f_{ij} is the empirical probability that a scallop with a shell height in the jth bin will grow into the ith bin in a year, assuming that the growth rings are produced annually. The matrix $\mathbf{F} = [f_{ij}]$ is a probability transition matrix, so that if $\mathbf{s}_y$ is the observed normalized size frequency of scallops at a given site in year y (grouped into the same size bins as the matrix and expressed as a column vector), then $\mathbf{s}_{y+1} = \mathbf{F}\mathbf{s}_y$ gives the predicted normalized size frequency one year later, excluding new recruitment. If the size frequencies that are forecasted using this method are close to the ones actually observed, then it can be concluded that the growth rings are laid down annually. In some cases, size frequencies were not available for certain years, so multiyear projections were made by applying the matrix to the original size frequency the appropriate number of times.

In the second method, we used the growth from the observed size frequencies to estimate the mean and variance of stochastic von Bertalanffy growth parameters for each site. These parameters were then used to predict the distribution of annual growth increments. The growth increments inferred from the size frequencies were then compared with those observed on the shells to test whether they were consistent with annual formation of the shell rings.

The von Bertalanffy model can be reformulated to predict the growth increment from a starting shell height (or length, L):

$$(1)\qquad \Delta L = (L_\infty - L)[1 - \exp(-K\Delta t)]$$

where ΔL is the predicted growth increment during time Δt (Fabens 1965). The parameters K and L_∞ can vary among individuals, even at the same site. For this reason, we will treat K and L_∞ as random variables. It will be assumed that K and L_∞ are independent with probability densities p_κ and p_{L_∞}.

Let C_L be the random variable representing the annual growth increment of an individual of starting size L. If K takes on the value κ, then, according to eq. 1, the value of L_∞ required to produce an increment of c starting at length L is $L + c/[1 - \exp(-\kappa)]$. Thus, p_{C_L}, the probability density function of C_L can be calculated as

$$(2)\qquad p_{C_L}(c) = \int_0^\infty p_K(\kappa)p_{L_\infty}\left(L + \frac{c}{1-\exp(-\kappa\Delta t)}\middle| L_\infty \geq L\right) d\kappa$$

The condition that $L_\infty \geq L$ is necessary because an individual observed at size L must have an L_∞ parameter greater than L (Bardos 2005). This condition will lead to greater growth increments at large sizes than would be predicted by deterministic von Bertalanffy growth.

If the sizes of the animals are measured into one of n size bins of width w with boundaries $h_0, h_1, \ldots, h_n$, then the probability that an individual that started in the jth size bin will grow into the ith bin ($i \geq j$) after time Δt is

$$(3)\qquad T_{ij}(\Delta t) = \frac{1}{h_j - h_{j-1}}\int_{h_{i-1}}^{h_i}\int_{h_{j-1}}^{h_j} p_{C_L}(x - L)\, dL\, dx$$

Now consider a fixed station that was sampled at m different times t_k ($k = 1, 2, \ldots, m$). The individuals in each of these samples were measured into one of n size bins. Let $B_k(i)$ ($i = 1, \ldots, n$; $k = 1, \ldots, m$) represent the number of individuals in the kth sample that were in the ith size bin, let $N_1, N_2, \ldots, N_m$ be the total number in each sample, and let $N_1', N_2', \ldots, N_m'$ be the number in the rth or larger size bins. Let $b_k(i) = B_k(i)/N_k$ be the normalized size frequency in the kth sample, and $b_k'(i) = B_k(i)/N_k'$ ($i \geq r$) be the normalized size frequency for scallops in the rth bin or larger. The integer r is chosen so that nearly all scallops from the previous year will be in the rth or greater bin, whereas almost all new recruits would be in the 1st through the $(r - 1)$th bins, so that our tracking of previously observed cohorts would not be confounded by new recruitment.

Based on the normalized size frequency in the $(k - 1)$th sample, the expected normalized size frequency in year t_k is

$$(4)\qquad p_k(i) = \sum_{j \leq i} T_{ij}(t_k - t_{k-1})b_{k-1}(j)$$

The log likelihood $\mathcal{L}_k$ that the population in the rth and larger size bins at time k was distributed into these bins in proportions $p_k(r), p_k(r + 1), \ldots, p_k(n)$ is given by the multinomial

$$(5)\qquad \mathcal{L}_k = \ln\left[\frac{N'!}{B_k(r)!B_k(r+1)!\ldots B_k(n)!}\right] + \sum_{i=r}^{n} B_k(i)\ln(p_k(i))$$

We assume that K and L_∞ are distributed as truncated (at 0) normals and thus are determined by their means and variances. The goal was to find the values of the means and variances of K and L_∞ at each site that maximize the total log-likelihood:

$$(6)\qquad \mathcal{L} = \sum_{k=2}^{m} \mathcal{L}_k$$

AD-model builder (Otter Research Ltd., Sidney, British Columbia) was used to estimate the means and the standard deviations of L_∞ and K that maximize the log-likelihood (eq. 6) based on the observed normalized shell heights $b_k(i)$ at each station. Growth increments were predicted from these estimates using eq. 2, which were then compared with those obtained by the shells.

Results

Comparisons of the size frequencies predicted using the growth transition matrices derived from the shell data with the observed size frequencies show a good fit in all but three of a total of 20 cases (Fig. 2; Supplemental Table S1[2]). One of these cases was a modest overestimation of growth from 2002 to 2004 at site 2 (Fig. 2*b*). More serious deviations occurred at site 4 (Fig. 2*d*), where nearly no growth was observed between 2005 and 2006, so that the size-frequency forecast from 2005 data overestimates the 2006 shell heights. A smaller discrepancy between forecast and observed growth also occurred at this site between 2007 and 2008. At site 3 (Fig. 2*c*), substantial new recruitment was observed in 2000, but when this recruitment was excluded and the size frequency was renormalized to reflect only the cohort observed in 1999, the projected and observed size frequencies were similar.

Mean growth increments based on estimated growth parameters from the size frequencies were similar to the mean growth increments from the shell rings (Fig. 3), with a large majority of the shell-based mean increments falling within 1 standard error of the mean (SEM) as estimated by the size frequencies. At site 1, the mean growth increments estimated from the size frequencies matched the mean shell growth increments nearly precisely. At site 2, shell increments tended to be somewhat smaller than those derived

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[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3920. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/eng/ibp/cisti/collection/unpublished-data.html.

Fig. 2. Comparison of observed relative size frequency (solid lines) with that projected from the previous observation (broken lines) using the growth transition matrix derived from the the shell rings. (*a*) Site 1 in the years 2001–2003 and 2005; (*b*) site 2 in the years 2002 and 2004–2006; (*c*) site 3 in the years 2000 and 2003–2008; and (*d*) site 4 in the years 2004–2008. All projections were based on the previous year, with the exception of site 1 in 2005 and site 2 in 2002 and 2004, which were based on two years previous, and site 3 in 2003, which was based on the size frequency observed three years previous.

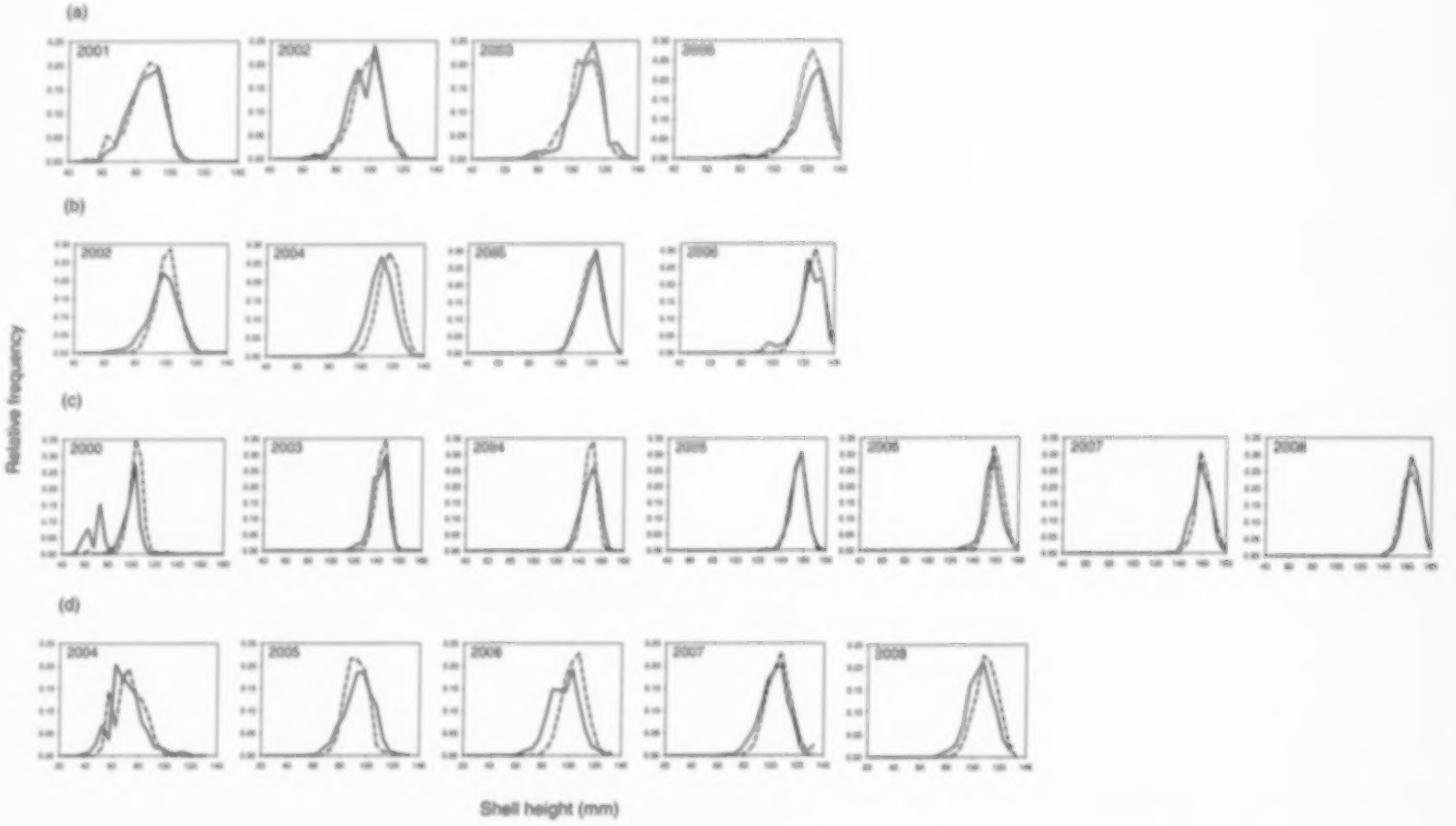


Fig. 3. Comparison of model-based predicted mean growth increments based on the size-frequency data (solid line) with mean growth increments in each 5 mm shell height bin from the shells (□). The broken lines show the model-based mean ± 1 standard error of the mean.

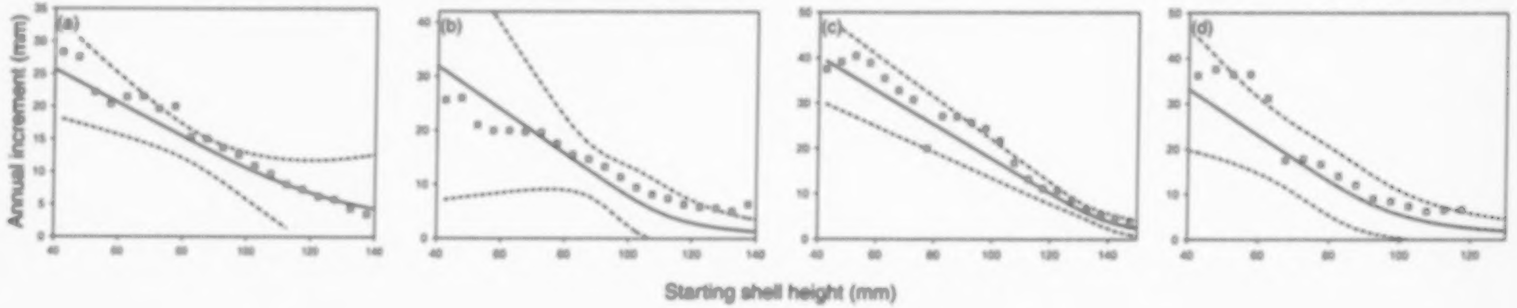


Fig. 4. Comparison at the mid-Atlantic site 4 of the observed relative size frequency (solid line) with that projected from the previous year using the growth transition matrix based on the shell rings under the alternative hypothesis that the rings are laid down twice yearly (Krantz et al. 1984). Comparisons are made for the years 2004 through 2008.

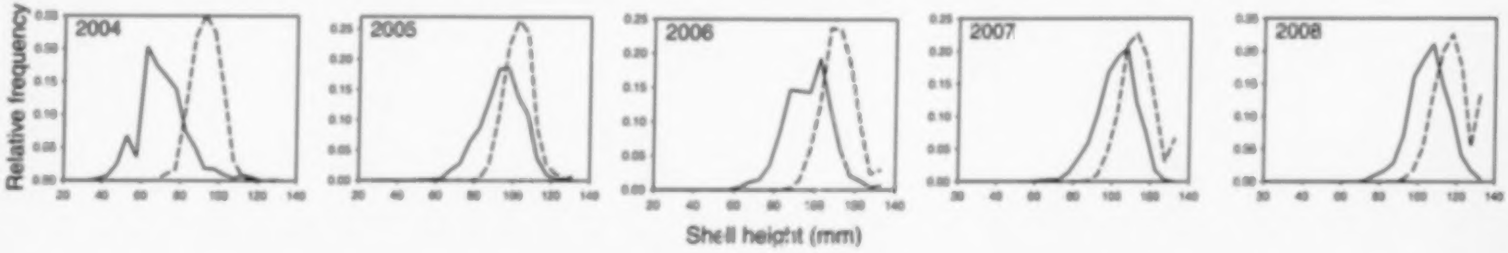
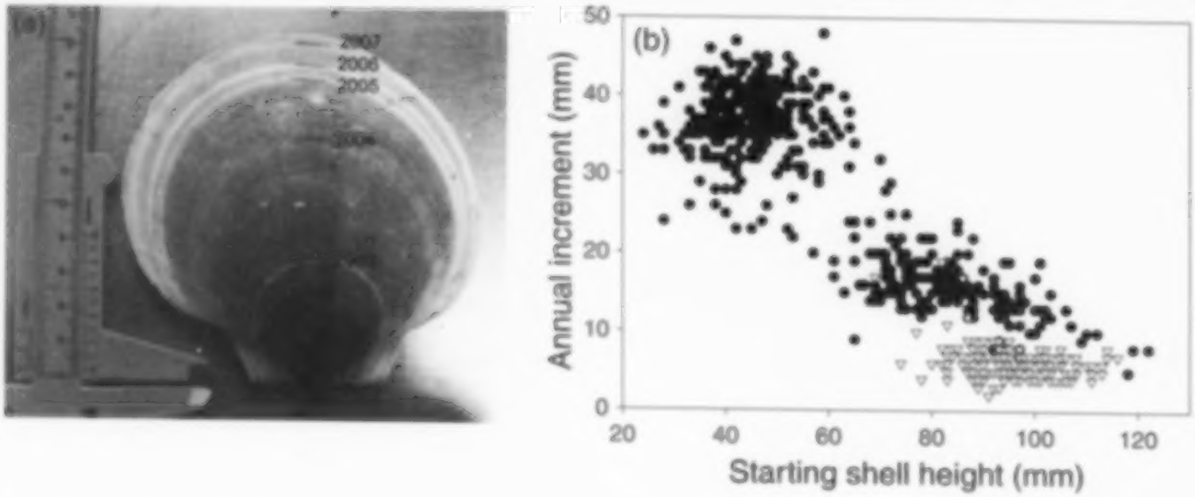


Fig. 5. (*a*) Photograph of a scallop shell collected at site 4 in 2008. The growth rings are marked with a pencil, and the years that the rings were formed are indicated under the assumption of annual ring formation. Note the narrow growth increment between 2005 and 2006. (*b*) Annual growth increments inferred from the shell rings vs. starting shell height for increments laid down prior to (●) and after (▽) 2005 at site 4.



from the size frequencies for small shell heights and somewhat greater for large shell heights. This may be caused by a slight underestimation of the mean L_∞ and a corresponding overestimation of the mean K from the size frequencies relative to the shell increments. At site 3, the predicted mean increments from the size frequencies were close to those from the shells, although the shell increments tended to be slightly, and nonsignificantly, greater than those inferred from the size frequencies. At site 4, the growth increments obtained from the shells were somewhat greater than those from the size frequencies, so that the shell increments suggested slightly faster growth than did the information from the size frequencies. However, even at station 4, most of the mean shell increments were well within 1 SEM increment derived from the size frequencies, indicating no statistically significant difference between the two. The close correspondence between the growth increments predicted from the growth observed from the size frequencies and the mean increments from the shells is especially noteworthy as they were obtained from independent sources of data.

Krantz et al. (1984) suggested that sea scallops lay down two growth rings annually in the mid-Atlantic, perhaps because of twice-yearly spawning. To test this idea at the mid-Atlantic site (site 4), forecasts were made based on this alternative hypothesis, so that the probability transition matrix from the shell increments was applied twice to each size frequency to predict the one for the following year (Fig. 4). Projected growth based on the Krantz et al. (1984) hypothesis always substantially overestimated the observed growth, indicating that this hypothesis is incorrect and that the growth rings are formed less often than twice yearly.

Discussion

Estimates of growth based on the shell rings matched the growth inferred from the size frequencies very well in most cases, indicating that sea scallop shell rings are annual and can be used to estimate growth. The one clear exception to this is at site 4 between 2005 and 2006, where there was little observed growth. Our methodologies assume that growth at shell height is stationary over time at each site. It is clear from the size frequencies that this assumption is violated at site 4, where rapid growth was observed between 2003 and 2005, followed by very limited growth between 2005 and 2006, and then moderate growth between 2006 and 2008. Growth estimated from the shell rings is an average over all years and thus would be expected to be an overestimate during the years when growth is slowest. It is not surprising that the shell increments predict somewhat greater average growth at this site than that from the size frequencies. Only the shells collected in 2007 and 2008 would have recorded the 2005–2006 slow growth period, whereas the fast growth observed between 2003 and 2004 would be observed on all the shells collected in 2005 through 2008. Thus, the mean growth increment based on the shells would be overweighted towards the early fast growth periods and hence would predict somewhat faster growth.

By counting back from the edge of the shell, it is possible to identify growth increments by year, assuming that they are formed annually. Consistent with the observed growth, most shells from site 4 show slower growth since 2005 and especially slow growth during the 2005–2006 period (Fig. 5*a*). The slope and intercept increment vs. starting shell height regressions were significantly different from the early (2003–2005) increments compared with the later (2005–2007) ones (Fig. 5*b*; $p < 0.001$ for both the slope and intercept), indicating a change in growth between these two periods. Thus, both the shell rings and the direct observations indicate that there was substantial temporal variability in growth, and the difference between the projected and observed size frequencies was due to this effect rather than to any inconsistency between growth inferred by the shells and observed growth.

Our work is not consistent with the hypothesis of Krantz et al. (1984), who suggested that two shell rings are formed yearly, at least in the mid-Atlantic. If even a nontrivial fraction of scallops laid down two rings annually, then growth would be underestimated under the assumption of annual ring formation. However, in none of the four sites did the shell-based growth analysis underestimate growth, and analysis based on the two rings per year assumption led to severely overestimated growth at the mid-Atlantic site. The Krantz et al. (1984) conclusions were based on stable isotope analysis of two scallop shells collected off Virginia. One of the shells analyzed was small (76 mm shell height). The visual identification suggested that this shell had just laid down its third ring (including the first year ring, which we have not attempted to identify), whereas the isotope analysis indicated that it was slightly less than 3 years old. This comparison is more consistent with annual than twice-yearly ring formation, so that their hypothesis was supported by a single shell only. The stable isotope ratio from the shells can depend on factors such as the drill depth of the sample, so that any conclusions based on a single shell may be unreliable. Alternatively, Krantz et al. (1984) may have erroneously identified rings on this shell that were not true annuli.

Absolute ages can be difficult to infer from sea scallop shells because no clear ring is laid down during the first one or more years. However, the shells do preserve a record of annual growth from the time of the first distinct visible growth ring. This allowed us to construct growth transition matrices directly from the shell ring data. This method does not require an assumption regarding the form of growth (in particular, von Bertalanffy growth was not assumed) and takes into account variability in growth among individuals in the population. One disadvantage to this nonparametric method is that large amounts of data are required to accurately populate the matrix entries. For several of our matrices, the right-hand columns were based only on the few sufficiently large shells at each site. In cases such as this where there are limited data, data smoothing and interpolation might be considered. Alternatively, the shell growth data could be used to estimate parameters of a growth curve, such as a stochastic version of the von Bertalanffy growth equation, which could then be used to generate the growth matrix. Stochastic growth matrices can be important components of size-based assessment methods, such as the CASA (catch-at-size analysis) model (Sullivan et al. 1990), and matrices based on shell increments were used in the 2007 assessment of US sea scallop populations (NEFSC 2007).

Our other method of estimating stochastic growth that estimates the mean and standard deviations of the von Bertalanffy growth parameters K and L_∞ from size-frequency data may also be broadly useful. Using observed growth from size frequencies to estimate growth parameters is often employed in species that are difficult to age, such as many invertebrates. Unlike many other methodologies (e.g., Fournier et al. 1990; Laslett et al. 2004), our method does not require a decomposition of the size frequencies into cohorts, which may be problematic in some cases. Because individual growth variability is explicitly modeled, our method avoids biases that often occur with methods that assume deterministic growth models (Sainsbury 1980; Bardos 2005; Eveson et al. 2007). In particular, deterministic von Bertalanffy growth predicts that a plot of annual increment vs. size at the beginning of the increment will be linear, with x intercept L_∞, leading to negative predicted growth for increments with starting size greater than L_∞. By contrast, our stochastic growth model (eq. 2) always predicts positive growth increments and substantial nonlinearities in increment vs. size plots for large sizes.

Both methods require minimal assumptions regarding recruitment and mortality. The only assumption about recruitment was that new recruits fall in the first $r - 1$ bins, which were then ignored in the fitting procedures. Sensitivity analysis indicates that value of r has little effect on the estimates; this is not surprising given that with a single exception (site 3 in 2000), new recruitment was negligible. The only assumption regarding mortality is that it was equal across size classes. In this respect, the fact that our study sites were located in areas closed to scallop fishing was important, as it precluded size-selective fishing mortality. Additionally, the lack of fishing mortality allowed a high proportion of the scallops to approach their asymptotic size, facilitating the estimation of L_∞. Our method of estimating growth from size frequencies observed in areas closed to fishing may be useful for many species provided that movement between the closed and fished areas is minimal, as is the case with sea scallops.

Our work demonstrates that sea scallop shell growth rings are laid down annually, corroborating most (Stevenson and Dickie 1954; Merrill et al. 1966), but not all (Krantz et al. 1984), previous work. Annual growth marks on shells have also been demonstrated in other scallop species, as well as a number of other types of bivalves (e.g., Murawski et al. 1982; Jones et al. 1983; Lomovasky et al. 2007). Given its veracity, visual analysis of shell rings is a rapid and inexpensive method of obtaining growth information.

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The North Atlantic subpolar gyre regulates the spawning distribution of blue whiting (*Micromesistius poutassou*)

Hjálmar Hátún, Mark R. Payne, and Jan Arge Jacobsen

Abstract: The spawning stock of blue whiting (*Micromesistius poutassou*), an economically important pelagic gadoid in the North Atlantic Ocean, increased threefold after 1995. The reproductive success of the stock is largely determined during the very early stages of life, but little is known about the spawning dynamics of this species. Here we show that the spawning distribution of blue whiting is variable, regulated by the hydrography west of the British Isles. When the North Atlantic subpolar gyre is strong and spreads its cold, fresh water masses east over Rockall Plateau, the spawning is constrained along the European continental slope and in a southerly position near Porcupine Bank. When the gyre is weak and conditions are relatively saline and warm, the spawning distribution moves northwards along the slope and especially westwards covering Rockall Plateau. The apparent link between the spawning distribution and the subpolar gyre is the first step towards understanding the reproduction variability, which currently is the main challenge for appropriate management of the blue whiting stock.

Résumé : Le stock reproducteur du poutassou (*Micromesistius poutassou*), un gadoïde pélagique d'importance économique du nord de l'Atlantique, a augmenté d'un facteur de trois après 1995. Le succès reproductif du stock est en grande partie déterminé durant les tout premiers stades de la vie, mais la dynamique de la fraie de l'espèce reste peu connue. Nous montrons ici que la répartition de la fraie du poutassou est variable et déterminée par l'hydrographie à l'ouest des Îles Britanniques. Lorsque le tourbillon subpolaire de l'Atlantique nord est puissant et répand ses masses d'eau froide et douce sur le plateau de Rockall, la fraie est confinée à la pente continentale européenne et à une région australe près du banc de Porcupine. Lorsque le tourbillon est faible et les conditions relativement salines et chaudes, la répartition de la fraie de déplace vers le nord le long de la pente et surtout vers l'ouest pour couvrir le plateau de Rockall. Le lien apparent entre la répartition de la fraie et le tourbillon subpolaire est un premier élément pour comprendre la variabilité de la reproduction, qui est actuellement l'obstacle principal pour la gestion appropriée du stock de poutassous.

[Traduit par la Rédaction]

Introduction

The northern blue whiting (*Micromesistius poutassou*) is a pelagic gadoid occupying mainly the waters between the spawning grounds west of the British Isles and the feeding areas in the Norwegian Sea (Bailey 1982). The spawning stock of blue whiting has increased dramatically in the late 1990s (Fig. 1) due to a succession of strong or extremely strong year classes (ICES 2007). The fishery intensified correspondingly and in recent years has been one of the largest fisheries in the North Atlantic Ocean (ICES 2007). Concurrent large changes have occurred in the ocean circulation in the northeastern Atlantic (Hakkinen and Rhines 2004), resulting in a rapid temperature and salinity increase west of the British Isles after the mid-1990s (Hatun et al. 2005; Fig. 1 and Supplemental Fig. S1[2]). Noting the tight temporal correlation between these events, we here ask whether the changes in marine environment and the blue whiting stock are causally related.

Possible causal relations are probably complex, as the environment influences many ecological components simultaneously. Blue whiting are sensitive to temperatures and salinities during the spawning period (Schmidt 1909), and

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[2] Supplementary data for this article are available on the journal Web site (http://cjfas.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Building M-55, 1200 Montreal Road, Ottawa, ON K1A 0R6, Canada. DUD 3919. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/eng/ibp/cisti/collection/unpublished-data.html.

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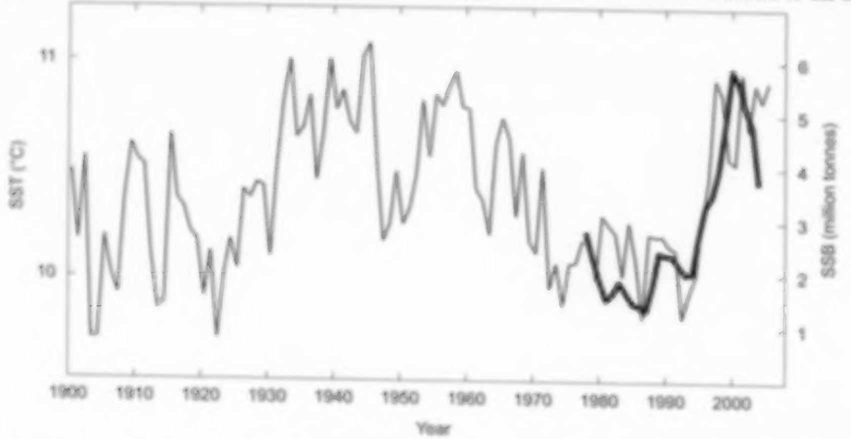
Fig. 1. Changes in the sea surface temperature (SST) in the northeastern Atlantic (thin line) and in the spawning stock biomass (SSB) of blue whiting (thick line). The annually averaged SST time series is obtained from the Hadley Centre SST data set (HadSST2) (Rayner et al. 2006) by averaging over a geographical box covering the northeastern Atlantic (52.5°N–62.5°N, 27.5°W–12.5°W). The SSB time series (ICES 2007) has been shifted 3 years forward in time, which is the time required for new recruits to contribute to the stock biomass.



the main spawning grounds are located west of the British Isles (Fig. 2), where large oceanographic changes are known to take place (Holliday 2003).

The water masses of the northeastern Atlantic at different depths have different origins with their own recognizable planktonic fauna or flora, according to their physical, chemical, biogeochemical, or biological features (Fraser 1961). The subpolar gyre controls the flow trajectory of the North Atlantic Current (NAC) in the northeastern Atlantic. When the gyre is strong and extends far eastwards, a branch of the NAC peels off into Rockall Trough, dragging cold, low-salinity subarctic waters over Rockall Plateau (Fig. 3*a*). When the gyre weakens and shifts westwards, it allows subtropical water to spread north and west (Fig. 3*b*), resulting in much warmer and more saline conditions. The strength of the subpolar gyre, and thus the hydrographic conditions in the Rockall region, has been represented by a so-called "gyre index" (Hatun et al. 2005; Supplemental Fig. S1[2]).

Because blue whiting are sensitive to hydrography while spawning in a region characterized by large water-mass exchanges, one might expect that the marine climate must influence the spawning distribution of blue whiting. The blue whiting fishery has historically been most intensive along the European continental slope, primarily near Porcupine Bank, but also west of the Hebrides (as illustrated in Fig. 2). These areas are typically thought of as the main spawning grounds of blue whiting (Monstad 2004). However, investigations of blue whiting larvae before the fishery commenced in the late 1970s indicated, on the other hand, that Rockall Plateau was the principal spawning ground, as opposed to Porcupine Bank (Henderson 1957; Fig. 2). Thus, there are apparent conflicting statements about the spawning areas of blue whiting and only limited explanations for their spawning dynamics (Bartsch and Coombs 1997; Skogen et al. 1999).

The dominance of Rockall Plateau as a spawning ground was particularly pronounced during the warm early 1960s, but the spawning distribution shifted southwards towards Porcupine Bank during the late 1960s (Bainbridge and Cooper 1973) when temperatures decreased in the region (Fig. 1). The stark contrast between the spawning distributions reported during the mid-19th century warm period and the cold, early 1990s (Fig. 2) suggests a link between the marine environment and the spawning dynamics of blue whiting. Bainbridge and Cooper (1973) postulated that the source of this variable distribution probably involved environmental variables, that the source of this variability might be found in regions remote from the spawning areas, and that they might affect the stock after a considerable time lag. They also stated that further progress on this topic had to await better environmental data over a longer run of years.

Since that work was published, we have witnessed the large mid-1990 changes in both the recruitment (and stock size) of blue whiting and the marine climate. Additionally, physical time series are now available that link the previous (1960s) warm period to the recent (post-1995) warm period. It is therefore timely to look at the previous and present spawning distributions and to collectively examine these in light of the oceanographic changes that have taken place.

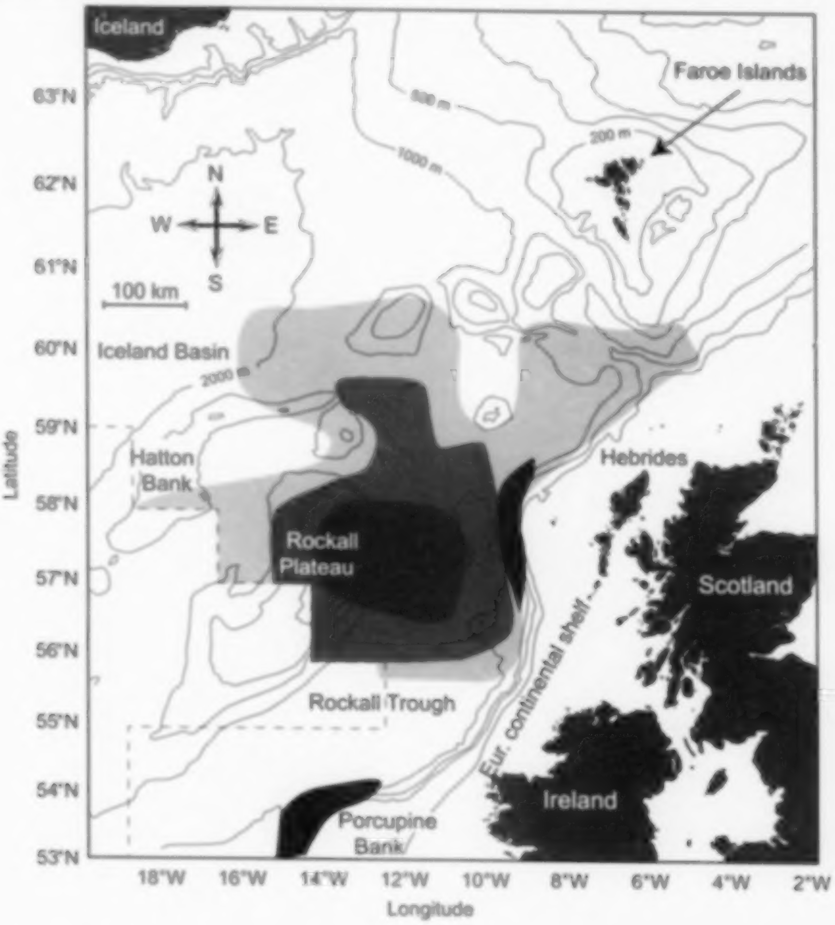
In trying to approach the fundamental question of a causal link between marine climate and blue whiting reproduction, we here limit the aim to document a link between the spawning distribution of blue whiting and the variable marine environment in this region. The working hypothesis that we explore here is that the spawning distribution is predominately controlled by the marine climate conditions to the west of Great Britain and Ireland, moving north along the European continental slope and west over Rockall Plateau during (saline and warm) periods when the subpolar gyre is weak, and south towards Porcupine Bank during (fresh and cold) periods when the gyre is strong.

Material and methods

The NISE hydrographic database

A comprehensive hydrographic (temperature and salinity) data set has been compiled in association with the project

Fig. 2. Map of the study region indicating places referred to in the text. The shaded contours illustrate the mean density of blue whiting larvae during the years 1948–1956 as reported by Henderson (1961). No larvae data are available in the region west of the broken line (southern part of the Rockall–Hatton Plateau). The intensive spawning areas during the 1980s and early 1990s, as reported by Monstad (2004), are shown in black along the European continental slope.



Norwegian and Iceland Seas Experiment (NISE). The basis of this data set is the public database maintained by the International Council for the Exploration of the Seas (ICES; www.ices.dk). This database is then supplemented by data from the Marine Research Institute (Iceland), the Institute of Marine Research (Norway), the Faroese Fisheries Laboratory, the Arctic and Antarctic Research Institute (Russia), the Geophysical Institute, University of Bergen (Norway), and the World Ocean Circulation Experiment (WOCE; www.woce.org). The data used here (post-1980) are generally measured by conductivity–temperature–depth (CTD) sondes, but data from the Argo float array (www.usgodae.org) have also been included. Data quality control has been performed by each data contributor, but some additional data cleaning has been applied to the data set by NISE (Nilsen et al. 2008).

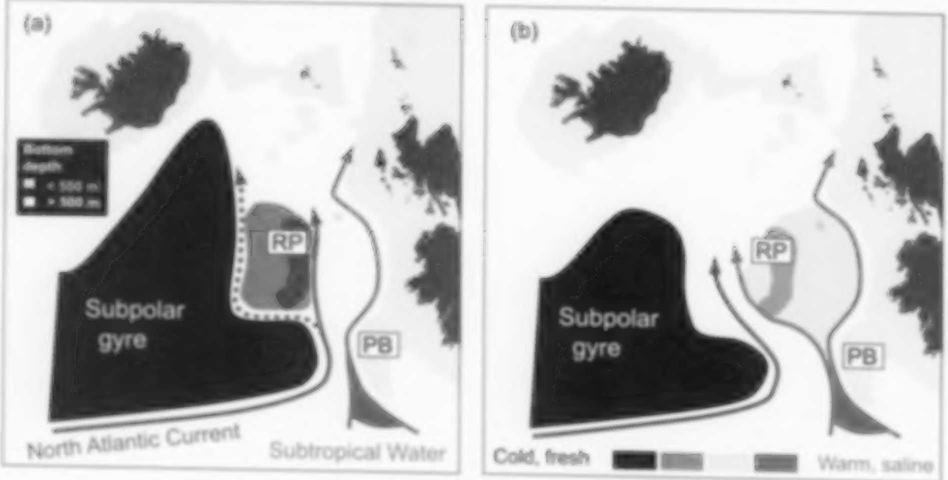
Acoustic biomass survey data

Acoustic abundance estimates of blue whiting in the spawning areas from 1981 onwards were obtained from the annual blue whiting surveys reported in the ICES "Northern Pelagic and Blue Whiting Working Group" reports (ICES 2007) as biomass averages (tonnes) over 0.5° latitude × 1° longitude rectangles (1981–2001).

During the standardized surveys, continuous acoustic recordings of fish and plankton were collected along the cruise tracks using calibrated 38 kHz echosounder systems. The acoustic recordings (area backscattering by each nautical mile, s_A) were allocated to blue whiting based on the composition of the trawl catches taken whenever needed to identify the acoustic scatterers, as well as by visual scrutiny of the echorecordings, whereafter biomass estimates were calculated by multiplying abundance in numbers by the average weight of the fish in each statistical square (Foote 1987; Toresen et al. 1998).

The annual surveys were carried out from mid-March to mid-April, with less than 2 weeks difference in timing. The error introduced by a variable timing in relation to the

Fig. 3. Simplified illustration of the source flows to the Rockall region. (*a*) A strong subpolar gyre results in strong influence of cold, subarctic water near Rockall Plateau (subarctic state). (*b*) A weak gyre results in a warm, subtropical anomaly near Rockall Plateau (based on Hatun et al. 2005) (subtropical state). Abbreviations: RP, Rockall Plateau; PB, Porcupine Bank.



spawning progress should be minor compared with the large spatial shift revealed by these data. The surveys are widely regarded as representing the actual spawning distribution and not the pre- or post-spawning migrations. The most recent data (after 1995) originate from joint international surveys, whereas the older data are obtained predominately from Norwegian surveys, with 2 years from Russian surveys.

Furthermore, the fish present in the area at spawning time are mostly actively spawning blue whiting. The proportions of immature fish in the spawning area varied in most of the years between nil and 10%, with some exceptions, e.g., around 15% in 2001, 1998, and 1989, mainly due to rich year classes contributing as young fish (1 or 2 group) in the area (ICES 2007). The survey coverage included the Porcupine Bank area, the shelf edge west of the Hebrides, in all years except 1982, 1997, and 2001. The Rockall Plateau area has been regularly covered since 1998, but only occasionally during the 1980s and early 1990s (Fig. 4*a*).

Blue whiting larvae data

Blue whiting larvae data were obtained from the Continuous Plankton Recorder (CPR) survey (Reid et al. 2003). The survey is a monitoring programme using a high-speed plankton sampling machine that is towed at 10 m depths behind ships of opportunity on standard routes during each month of the year. The data presented here are obtained from tows performed by ocean weather ships patrolling the waters west of the British Isles from 1951 to 1970 (Bainbridge and Cooper 1973). These data have kindly been reworked and made available to us by the Sir Alister Hardy Foundation for Ocean Science (www.sahfos.ac.uk).

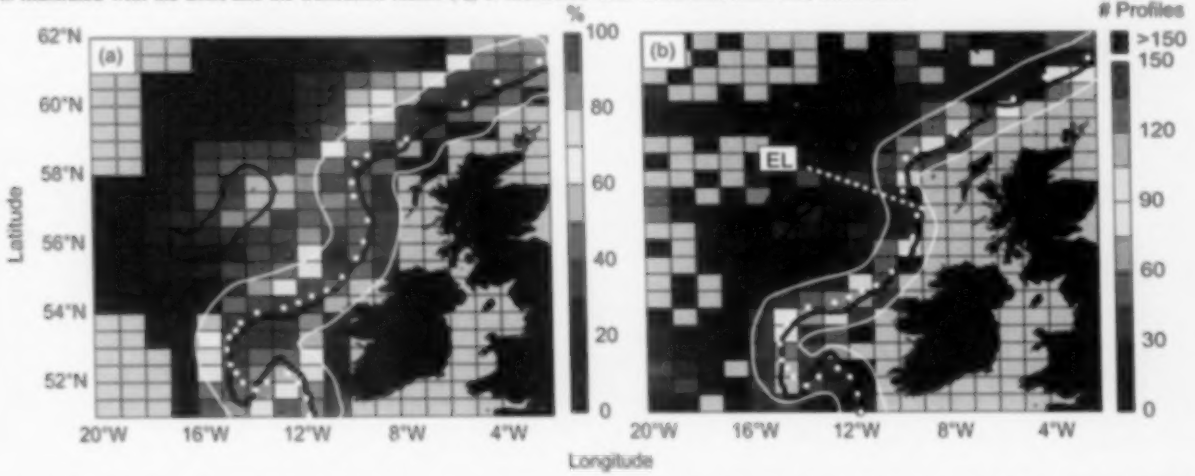
Fisheries catch statistics

Under the North Eastern Atlantic Fisheries Commission (NEAFC; www.neafc.com) scheme, vessels report to their respective (national) contracting parties and this information is shared via the NEAFC Secretariat's database. The blue whiting catch statistics applied here are obtained from this database. The data are available as monthly values of the total catch gridded onto 0.5° latitude × 1° longitude rectangles and differentiated for each national fleet. Data from the Norwegian fleet are used exclusively, as the spatial distribution of this fleet was not limited by political regulations. The other major fishing nations for blue whiting, the Faroe Islands and Russia, had only limited or no access, respectively, to European Union waters during most of the period from 1980 to 2007. The fishery in spring is focused on spawning blue whiting. We assume that the figures given represent catches of adult spawning blue whiting with proportions of immature fish comparable with those in the surveys reported above, as the fishery tend to selectively concentrate on large (adult) and, thus, mature fish.

Regional gyre index

A regional gyre index, based on simulated sea surface height, has been produced here. This is done by repeating the analysis of Hatun et al. (2005), while limiting the analysis to a geographical region in the northeastern Atlantic Ocean (54°N–66°N, 30°W–0°W). The regional index presented here is similar to the one presented by Hatun et al. (2005), with the exception of a dip in the mid-1980s, which is largely ascribed to dynamics in the Irminger and Labrador seas. The employed numerical Ocean General Circulation Model (OGCM) is the Nansen Center version (Bentsen et al. 2004; Drange et al. 2005) of the Miami Isopycnal Coordinate Ocean Model (MICOM; Bleck et al. 1992), forced with daily mean National Centres for Environmental Prediction / National Center for Atmospheric Research (NCEP/NCAR) (Kalnay et al. 1996) reanalyses of fresh water, heat, and momentum fluxes for the period 1948–2003. Because this model system terminated in 2003, the gyre index has been extended to 2006 using satellite altimetry (Hakkinen and Rhines 2004, updated).

Fig. 4. Data coverage and the *s* axis. (*a*) Spatial coverage of acoustic surveys (1981–2007) showing the fraction coverage of each 0.5° latitude × 0.5° longitude square and (*b*) number of hydrographic stations with available data at 300 m depths during the 1981–2007 period, binned into the same geographical squares. Squares with no acoustical data or fewer than five hydrographic stations are shaded. The *s* axis is illustrated with the dots, and the truncation radius (r_0) is illustrated with white thin lines. EL, Ellett Line.



Use of spatial data transformations

The biological and physical data sets described above are generally scattered in both time and space, with variable resolution, making direct comparisons difficult. The survey biomass and the hydrographic data are most densely distributed along the European continental slope (roughly meridional) (Fig. 4), whereas the CPR larvae data are most densely distributed along track lines fanning out westwards from the continental slope towards Hatton Bank (Bainbridge and Cooper 1973). To enable meaningful comparisons, each data set has been transformed onto a common axis, following the regions with densest data coverage.

To illustrate meridional shifts in the survey biomass and the hydrography along the European continental slope, these data have been transformed onto a curvilinear axis (*s* axis) tracking the high density of observations along the continental shelf edge, starting within Porcupine Seabight in the south (50.5°N) and continuing with points along the slope north to 61°N, west of Shetland (Fig. 4). To illustrate blue whiting larvae distribution shifts on–off Rockall Plateau, the CPR larvae data are transformed onto a rectilinear axis (*r* axis) extending from the continental slope northwestward towards Hatton Bank.

The value at a given position *x* along these axes is calculated as a weighted average of the available observations within a truncation radius (r_0) around this position. If too few observations are available near a position *x*, the truncation leaves the transformed value void, instead of having it represented by only a few too remotely positioned observations. The transformed value at position *x* can be written as

$$(1)\qquad \hat{D}(x) = \frac{\sum_{i}^{N} D_i w(d_i)}{\sum_{i}^{N} w(d_i)}$$

where *N* is the number of observations (D_i) within the truncation radius r_0, and *w* is a weight, which is a function of the distance d_i from position *x*. A Gaussian weighting function was used, as it gives a smooth distribution and its properties are easily understood. This weight is given by

$$(2)\qquad w(d) = \exp\left(\frac{-d^2}{2\tau^2}\right)$$

where *d* is the distance between position *x* and the location of the observation, and τ is the width of the filter. Because we are interested in shifts in distributions more than the interannual abundance variations, the biological observations have been normalized by the annual along-axis mean.

Results

Meridional shifts

Survey biomass and the subpolar gyre

Meridional shifts in the concentration of spawning blue whiting along the European continental slope (*s* axis) are seen to co-vary with the dynamics of the subpolar gyre (Fig. 5*a*). For transforming the survey data onto the *s* axis, these biomass observations are regarded as data points located in the centre of each 0.5° latitude × 1° longitude rectangle, as presented in the ICES reports. The Gaussian filter width (τ) and the truncation radius (r_0) were both 100 km, as this is comparable with the size of each data rectangle. The denominator in eq. 1 removes the effect of high or low survey coverage in an area, and the obtained value is thus consistent and comparable along the entire *s* axis. This gives a normalized Gaussian-weighted mean density of blue whiting observed within a 200 km wide swath along the *s* axis, including the spawning period every year since 1981 (Fig. 5*a*).

The regional gyre index is plotted onto the survey biomass map (Fig. 5*a*, black curve), together with the salinity (red curve) observed along a section (Ellett Line; Fig. 4*b*) extending from the continental shelf towards Rockall Bank

Fig. 5. (*a*) Time and latitude or distance (Hovmüller) plot of normalized biomass density of blue whiting along the European continental slope (*s* axis). The thick black curve shows a gyre index based on the simulated sea surface height in the northeastern Atlantic, and the broken black curve shows an extension of this index based on satellite altimetry (Hakkinen and Rhines 2004, updated). The red curve shows salinity averaged over the depth range of 0 to 800 m at the Ellett Line (Holliday et al. 2000). Open areas indicate absence of survey data. (*b*) Hovmüller plot of salinity at 300 m depths along the *s* axis. Only data from March to June have been included. The salinity range (35.35–35.45 psu) encompassing the densest blue whiting observations in (*a*) is emphasized with thick black lines. Available data points are illustrated with small crosses.

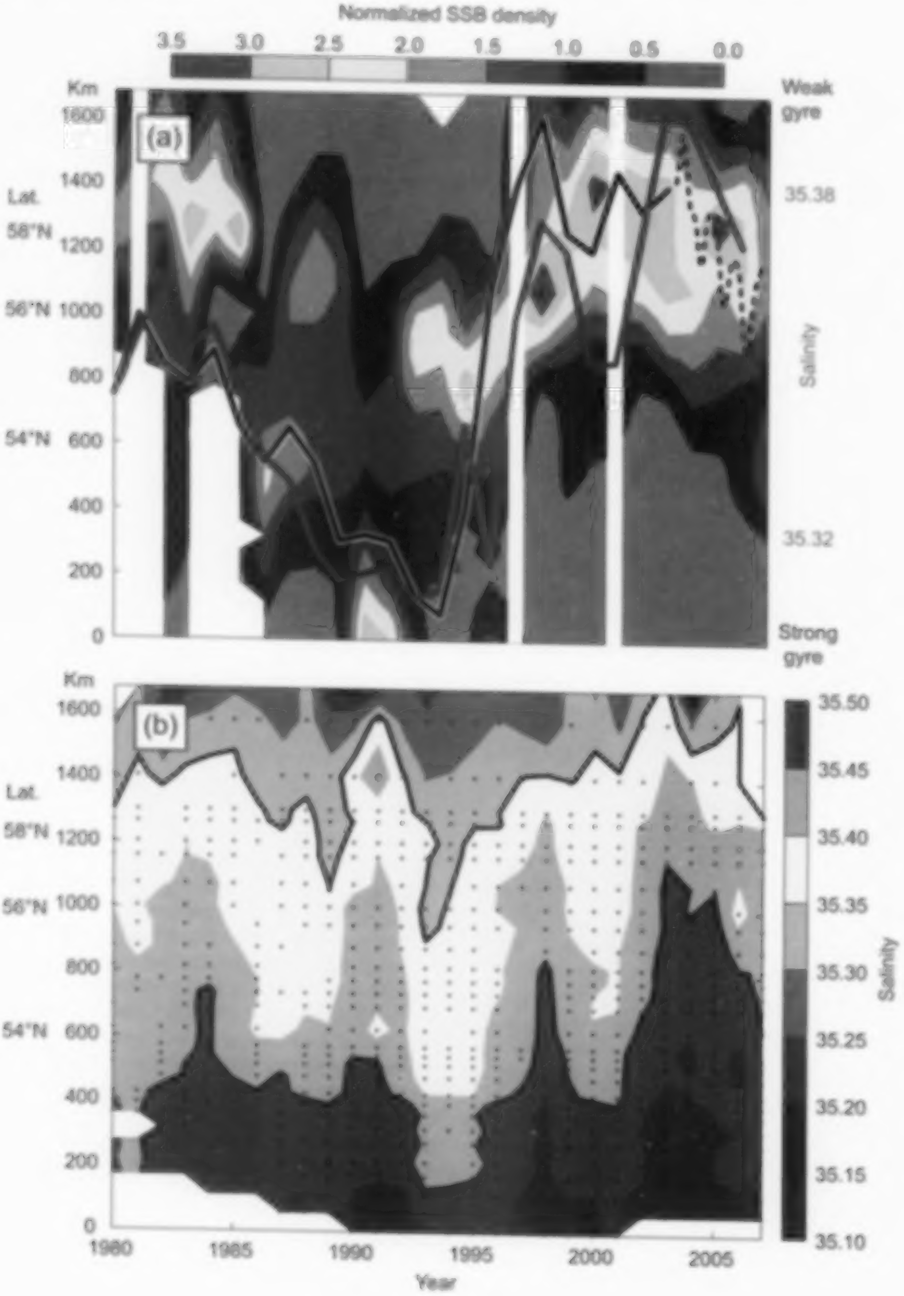
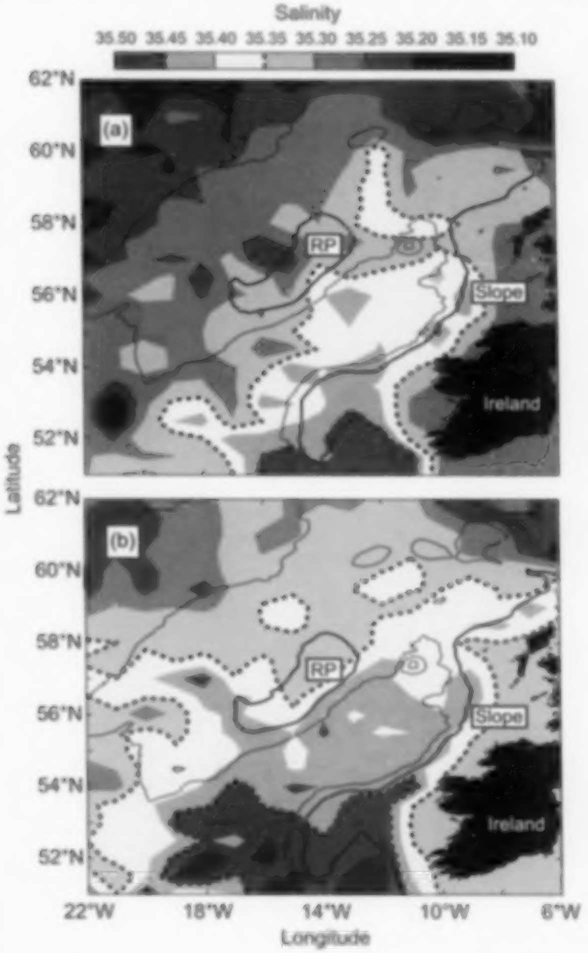


Fig. 6. March–June temperature composites at 300 m depths for (*a*) the fresh pre-1995 years (1986–1995) and (*b*) the saline post-1995 years (1996–2005). The 35.35 and 35.45 psu isohalines have been emphasized with thick black broken lines. RP, Rockall Plateau.



across the northern Rockall Trough (Holliday et al. 2000). The salinity is averaged over a depth range of 0 to 800 m, and the time series illustrates the strong subpolar gyre related exchanges of water masses around Rockall, as previously demonstrated (Hatun et al. 2005). Salinity is shown as this is a more conservative tracer than temperature and thus a better indicator for oceanic advection.

The gyre was relatively weak in the early 1980s, the salinities were high, and although survey coverage is incomplete during this time, the available observations suggest that spawning has a northerly orientation, taking place on the shelf edge west of the Hebrides (~58°N) (Fig. 5*a*). The gyre intensified appreciably in the early 1990s, resulting in very low salinities and the largest spawning concentrations in Porcupine Seabight, to the far south. The sudden weakening of the subpolar gyre after 1995 enforced a rapid increase in salinities and a clear northward shift of spawning activity. Since about 2003, the gyre has intensified slightly, salinities have again decreased, and the spawning distribution has retracted somewhat toward the south. Statistical analysis shows significant correlations between the location of the maximum spawning concentrations on the *s* axis and both the gyre index ($r = -0.56$, df = 15, $p = 0.021$) and the average salinity on Ellett Line ($r = 0.62$, df = 15, $p = 0.007$). Similar correlations are seen using the centre of mass of the distribution as the dependent variable instead of the location of the maximum.

Hydrographic threshold

The largest concentrations of spawning blue whiting are observed within specific salinity (Fig. 5*b*) and temperature (Supplemental Fig. S2)[2] ranges. Demonstrating this requires considerable hydrographic data distributed along the southward migration route of the prespawners. These data are not available over the open ocean areas (Fig. 4*b*), and the analysis has therefore been done along the *s* axis, bearing in mind that this might not entirely represent the environment experienced by the migrating fish.

The hydrographic data from the spawning period (March to June) were first interpolated onto 300 m depths using the software Ocean Data View (Schlitzer 2007). The salinity at 300 m, considered as representative of the environment experienced by spawning blue whiting (Bailey 1982), was then transformed onto the *s* axis. The result, however, was not sensitive to the choice of depth, as the water column is rather homogeneous after winter convection. The Gaussian filter width (τ) and the truncation radius (r_0) were both 50 km (see Fig. 4*b*). This gives salinities at 300 m depths, along the continental slope from 50°N to 61°N, for the spawning period every year since 1981, as shown in the time–longitude (Hovmüller) plot (Fig. 5*b*).

The isohalines move south until 1995, followed by a subsequent northwards shift, resembling the variability of the subpolar gyre and the survey biomass (Fig. 5*a*). This shift is clearest for the 35.35 to 35.45 psu isohalines (Fig. 5*b*, black curves). A second point to note is that the anomalies occur in pulses, one around 1990, one around 1997, and one around 2003. The two latter pulses have their counterpart in the gyre dynamics, whereas the pulse around 1990 does not.

A similar analysis has been performed using temperatures (Supplemental Fig. S2[2]), leading to similar conclusions: blue whiting prefer to spawn within relatively narrow ranges of salinity (35.35–35.45 psu) and temperature (9–10 °C), in gross agreement with previous findings (Henderson 1957; Schmidt 1909).

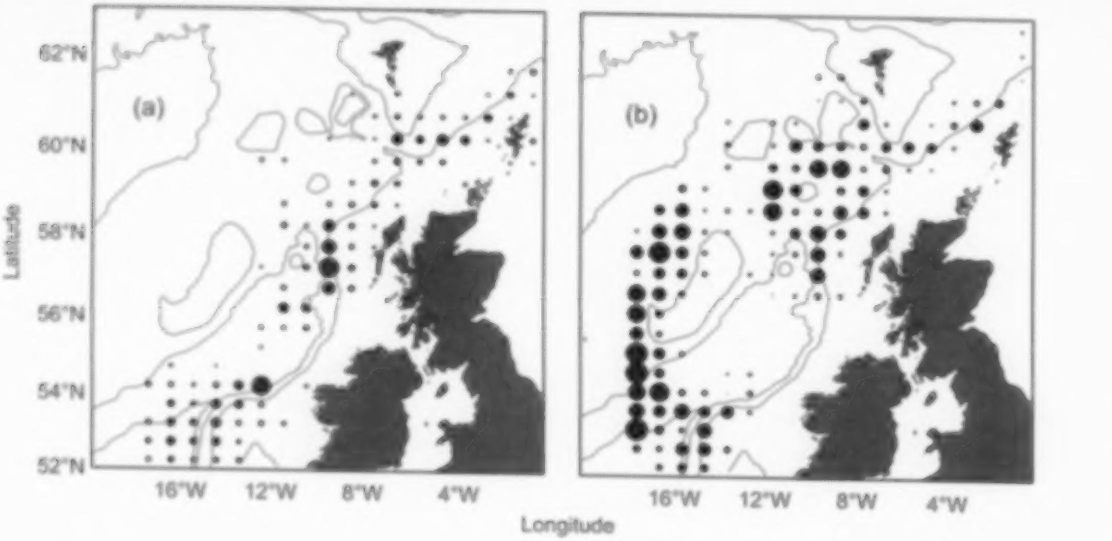
On–off Rockall Plateau

Hydrography

The above analysis suggests that a salinity range of 35.35 to 35.45 psu, a temperature range of 9 to 10 °C, or similar water-mass characteristics related to these hydrographic ranges govern the spawning distribution of blue whiting. If this result is generally valid, we would expect to see evidence of spawning activity throughout these water bodies, not just within the relatively narrow range covered by the ICES acoustic surveys considered here (Fig. 4*a*).

The mid-1990s weakening of the subpolar gyre (Hakkinen

Fig. 7. Distribution of adult catches of blue whiting reported by the Norwegian fleet, averaged over (*a*) the low-saline, cold years from 1989 to 1996 and (*b*) the saline, warmer years from 1997 to 2005. The area of each circle is proportional to the amount of blue whiting fished within a 0.5° latitude × 1° longitude rectangle centered on the dot.



and Rhines 2004) (Fig. 5*a*) represents, as mentioned, a particularly pronounced shift from a subarctic-influenced regime to a subtropical-influenced regime. This period is thus used to depict the variable dynamics of the system. Composite maps of salinities at 300 m for the low-saline pre-1995 decade (Fig. 6*a*) and the saline post-1995 decade (Fig. 6*b*) illustrate that the salinity changes are even larger around Rockall Plateau than near the continental shelf. The salinity interval (35.35–35.45 psu), when most spawning is observed near the continental slope (Fig. 5*b*), does not cover Rockall Plateau before 1995 (Fig. 6*a*) but does cover it during the decade thereafter (Fig. 6*b*). A similar statement can be made based on temperatures (Supplemental Fig. S3)[2]. Does this mean that blue whiting also spawned near the bank after 1995?

Fisheries

The distribution of the spawning fishery provides some insight into this question. A marked westward shift occurred in the fishing location of the Norwegian fleet during the mid-1990s (Fig. 7). No catches of blue whiting are reported west or southwest of Rockall Bank before 1996 (Fig. 7*a*), whereas very large catches are reported there after 1996 (Fig. 7*b*). The Norwegian fleet was not greatly limited by political regulations in the Rockall region and should therefore be a reasonably unbiased proxy for the distribution of spawning fish in this region. In the absence of more reliable biological observations of blue whiting, these data are an indication of significant spawning west of Rockall Plateau after the 1995 hydrographic shift.

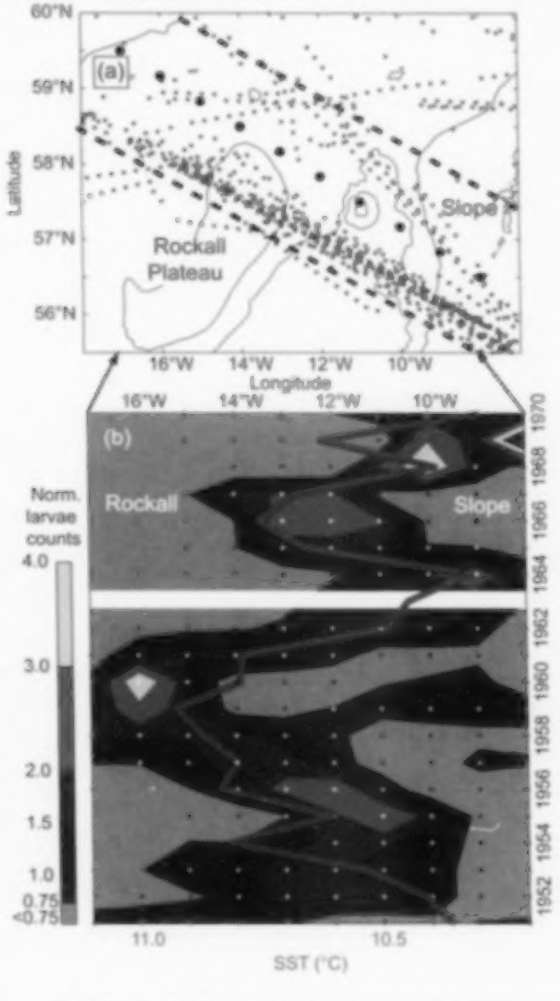
Rockall – continental slope larvae distribution and sea surface temperature (SST)

Historical observations of larvae from the CPR survey also provide insight into the presence of spawning over Rockall Plateau. CPR larvae data from the northern Rockall Trough during the period 1951–1970 have been transformed onto a linear axis (r axis) from the continental slope at 57°N extending northwestward towards Hatton Bank (Fig. 8*a*), using the Gaussian transformation scheme described above (eqs. 1 and 2). The Gaussian filter width (τ) and the truncation radius (r_0) were both 100 km (see Fig. 8*a*) so as to encompass a sufficient number of tows without spanning too wide. Only data from the spawning period March–June have been used. Currents in this region generally flow northwards parallel to the continental shelf (Fig. 3) (Ellett et al. 1986) and thus perpendicular to the r axis, so that drift between spawning and the observation of larvae is not expected to greatly alter the east–west distribution. The analyzed data show a clear east–west meandering of the larvae concentration evident in the longitude–time (Hovmüller) plot (Fig. 8*b*).

Salinity data in this region are sparse during this early period, and we are forced instead to use SST as a proxy of oceanographic conditions. Comparison with the simulated gyre index in recent times shows that SST represents, to first order, the oceanographic changes in the region (Supplemental Fig. S1)[2]. However, because of the spin-up period of the numerical ocean model (the integration of which started in 1948), the SST time series is considered to be a more reliable measure of oceanographic conditions for the early 1950s–1960s period.

When the SST time series is plotted onto the larvae map (red curve in Fig. 8*b*), good qualitative agreement is seen between the east–west distribution of the larvae and the average SST in this region. Warmer temperatures are seen to cause a westward shift in the larval distribution towards Rockall Plateau. Statistical analysis shows a significant correlation between the location of the maximum larvae concentrations on the r axis and the SST ($r = -0.50$, df = 18,

Fig. 8. (*a*) The *r* axis (●) and positions with larvae data from the CPR (red dots) during the period from 1951 to 1970. The truncation radius (r_0) is illustrated by the broken blue lines. (*b*) Longitude–time (Hovmüller) plot of the normalized larvae counts. The red line indicates the sea surface temperature (SST), as described in Fig. 1. Available data points are illustrated with small crosses.



p = 0.024). A similar correlation is seen using the centre of mass of the distribution as the dependent variable.

Discussion

Blue whiting distributions during a century

We here show that the spawning distribution of blue whiting is determined by the oceanographic conditions to the west of Great Britain and Ireland, which in turn are regulated by the North Atlantic subpolar gyre. A century after Schmidt (1909) made the first mapping of the blue whiting distribution, we have now begun to understand how climate and large-scale oceanography shifts and warps this distribution. The first mapping was made during the coldest years (1904–1908) of the 20th century, and Schmidt (1909) observed a "striking absence" of fry west of Rockall Bank, whereas significant amounts of fry were observed near Porcupine Bank. The distribution of fry observed with the CPR during the rapidly warming period from 1948 to 1955 contrasted with Schmidt's findings, as no fry were observed near Porcupine Bank, whereas large concentrations were observed around Rockall Plateau (Henderson 1957). The larvae concentrations decreased in the north and increased further south during the late 1960s, when the climate again deteriorated and temperatures declined (Bainbridge and Cooper 1973).

Using acoustic survey data along the continental slope, we have shown that spawning fish were aggregating at a northerly position during the early 1980s but shifted again southwards towards Porcupine Bank during the late 1980s and especially during the very fresh, cold early 1990s. After the dramatic decline of the subpolar gyre in 1995, the fish concentrations shifted about 1400 km northwards along the continental slope. The spawning distributions of blue whiting reported here and in the literature can therefore be better understood by putting them in a century-long hydroclimatic perspective. These hydroclimatic changes have been attributed to the subpolar gyre dynamics for the post-1960 period (Hatun et al. 2005), but the gyre forcing might also have been dominating during the pre-1960 period, although data are too scarce to show this.

On–off Rockall

In this study, we especially want to draw attention to rapid changes in the spawning distribution to include Rockall Plateau. Coverage by the acoustic surveys is inconsistent in this area, and no larvae data are available to document a possible sudden increase in spawning near Rockall after 1995. The fishery catch statistics, however, do indicate a marked shift after 1995.

Reanalyzing the blue whiting data presented by Bainbridge and Cooper (1973), while focusing on the Rockall Plateau – continental slope dimension, we showed marked on–off Rockall shifts during the two decades 1951–1970. Comparing this with the sea surface temperatures (SST) in the region revealed consistent near-Rockall distributions during warm years and a near-slope distribution during colder years. This result is consistent with our hypothesis of intensive spawning near Rockall during the warm post-1995 period. The apparent conflicting depictions of the spawning distribution of blue whiting presented by Henderson (1957) and Monstad (2004) can thus be explained by the fact that they represent warm and cold regimes, respectively.

Hydrographic escort

We have discussed the spawning dynamics in relation to temperature and salinity mostly because these parameters are widely available and facilitate a century-long perspective on the marine environment – blue whiting link. Henderson (1957) stated that a hypothesis of too high temperatures for spawning near Porcupine Bank during the 1950s was hardly tenable considering that the same fish species also spawns in the much warmer Mediterranean Sea. In this regard, it is important to understand that SST and salinity changes in the northeastern Atlantic are proxies for large water-mass exchanges. Such redistribution of water masses alters not only temperatures and salinities, but also chemistry and plankton contents in the ocean. The indication that most spawning occurs within narrow ranges of salinities (35.35–35.45 psu) and temperatures (9–10 °C) could therefore be an expression of prespawning fish seeking the chemical clues or the plankton composition contained by water masses with this temperature–salinity signature.

The spawning distribution, irrespective of the actual guidance mechanism, must be determined during the late winter period when the fish are migrating southward. An instructive example of how this may work is that of mackerel (*Scomber scombrus*), a pelagic species with spawning migration dynamics resembling that of blue whiting. For this species, it has been demonstrated that the southward migrating fish swim quickly when they are in cold water, but slow down when they encounter warmer waters (Reid et al. 1997). This characteristic has been called "enviroregulation" (Neill 1984). An evolutionary process may have "programmed" this fish species to deliver their eggs in a specific marine climate and fauna, which maximizes the survival of their offspring. If enviroregulation also governs the southward migration of blue whiting, this can explain the association between hydrography and spawning distribution. For example, the preferred hydrographic range does not encompass Rockall Plateau during the low-saline and cold pre-1995 decade, so prespawners located near the bank will continue to swim southeastwards along the salinity–temperature gradient, thus towards Porcupine Bank. Alternatively, during the post-1995 period the preferred hydrographic conditions cover Rockall Plateau; therefore, ripe fish first entering this region will not continue to swim southward, but rather start spawning.

A coherent picture from vague components

To illustrate both the long-term and large-scale changes in the spawning distribution of blue whiting, it has been necessary to utilize many widely differing data sources. Although each individual source does not provide conclusive evidence supporting our hypothesis, taken together, this material gives a consistent picture of the spawning dynamics.

The acoustic survey biomass is associated with uncertainties due to the acoustic method itself and the timing and spatial coverage of the cruises. Although individual years and details of the survey distribution might be unreliable, the characteristic southward shift until the mid-1990s, followed by a northward shift, is probably a real signal.

The similar pattern seen in the meridional shifts of isohalines along the continental slope are also believable, although data from both near-shelf and more-offshore locations have been included. The data coverage is sparse until 1987 but relatively good thereafter, as shown herein. The basic patterns discussed are not greatly influenced by data limitation after 1987. Aliasing due to the seasonal cycle is found to be insignificant as the seasonal salinity variability at 300 m is much smaller than the interannual variability. Using only data from late spring – early summer (March to June) further alleviates the aliasing problem.

The mid-1990 salinity changes at 300 m depths were illustrated by lumping scattered observations into a pre-1995 decade and a post-1995 decade and applying an objective mapping technique (Bohme and Send 2005). Although this is a crude depiction, it probably illustrates the northward flush of high-salinity water in a realistic way, due to the large extent of the mid-1990s salinity changes. We also feel that the extent of the mid-1990s changes in the fishery must be caused by a real shift in the spawning stock, despite the fact that fishery statistics often are strongly biased by misreporting and that the number and efficiency of the trawlers increased greatly during the mid-1990s.

Some doubt has been cast on how well larvae data represent spawning distribution, as the larvae may have drifted considerable distances since spawning (Bainbridge and Cooper 1973). Furthermore, the larvae data are sampled at 10 m depths and can be significantly influenced by aperture avoidance. The sampled routes towards the weather ships vary somewhat, and the approach of decomposing these data onto an axis (*r* axis) could therefore be questioned (Henderson 1957), but the close covariability between SST and the east–west meandering of the larvae distribution still lends credibility to the result. However, the very low larvae counts near the continental slope during warm years probably do not reflect an absence of spawning there. It more likely reflects a northerly spawning distribution and that the eggs may have been either spawned to the north of the *r* axis or already drifted north prior to the time of sampling.

Outlook and recruitment

Our ability to link the spawning distribution of blue whiting to the dynamics of the subpolar gyre is perhaps the most promising result in this study. Using realistic numerical ocean models, oceanographic databases, and satellite-based observations (Hakkinen and Rhines 2004; Hatun et al. 2005), it is now possible to better understand the dynamics of this gyre and its atmospheric forcing mechanisms (Eden and Willebrand 2001). These issues are not further elaborated here but will constitute the basis for future work aiming at a better understanding of the blue whiting stock dynamics.

Having established a link between the spawning distribution and the marine climate, the next challenges are to explain how the changeable spawning distribution might influence the recruitment to the blue whiting stock. The large shifts in the marine environment will probably influence larvae growth conditions near both the continental slope and Rockall Plateau. Assuming that the on–off Rockall Plateau variability of blue whiting spawning activity is the most important mechanism underlying the pronounced recruitment variability, we suggest the following causal relations: firstly, that eggs and fry starting on the plateau will follow different drift paths from those starting on the shelf that would enlarge and diversify their nursery area and thereby improve growth–survival opportunities of the larvae; secondly, that a potentially strong retention of water masses near Rockall Bank during warm low-gyre index years (Dooley 1984) might provide good local growth conditions; and thirdly, that larval development near Rockall might be in closer synchrony with production cycles of key prey types (zooplankton) than nearer the continental slope (Bainbridge and Cooper 1973).

Differentiating between these competing hypotheses is a challenging task that should be validated by (*i*) applying hydrographic drift modeling to investigate how the initial position of eggs and larvae might influence the downstream spread of larvae and the environment they encounter, (*ii*) establishing regular field studies to sample larvae growth–size and feeding intensity – food content simultaneously in the spawning areas and in the downstream larval feeding areas and relate this to the ambient prey availability and the hydrographic environment, and (*iii*) examining expected east–west shifts in the distribution of 0- and 1-year-old blue whiting by using annual demersal trawl surveys on the Icelandic, Faroese, and North Sea's plateaus (cf. Heino et al. 2008).

Such questions must be understood before reasonable predictions of the dynamics of the blue whiting stock in a changing environment, natural or man-made, can be made.

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A novel statistical method for validating the periodicity of vertebral growth band formation in elasmobranch fishes

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Abstract: A simple age verification method is presented for centrum edge analysis (CEA) of elasmobranch species. In this method, a binomial model is linked with a von Mises distribution for circular data, taking the characteristics of the CEA data into account. The periodicity of growth band pairs is categorized as no cycle, an annual cycle, or a biannual cycle. Three models are then constructed according to different periodicities. We use the Akaike information criterion (AIC) to determine which model is the best. The models were applied to the shortfin mako shark (*Isurus oxyrinchus*) data collected in the North Pacific to identify the best periodicity model. The AIC best-fitting model was one involving an annual cycle. The general performance of the method was evaluated using simulated data of various sample sizes, formation times, and durations of growth band pairs. The simulation trials showed that the performance of the method was satisfactory with moderate sample sizes. This method should improve the accuracy of age determination and could be applied to all species that have periodic growth band pairs.

Résumé : Nous présentons une méthode simple de vérification de l'âge dans l'analyse de la bordure du corps vertébral (CEA, « centrum edge analysis ») chez les espèces d'élasmobranches. Dans la méthode, un modèle binomial est relié à une distribution de von Mises pour des données circulaires qui tient compte des caractéristiques des données de CEA. La périodicité des paires de bandes de croissance est caractérisée comme non cyclique ou alors comme à cycle annuel ou bisannuel. Trois modèles sont ensuite construits en fonction des différentes périodicités. Le critère d'information d'Akaike (AIC) sert à déterminer le meilleur modèle. Les modèles ont été utilisés avec des données sur le mako à nageoires courtes (*Isurus oxyrinchus*) récoltées dans le Pacifique Nord afin de déterminer le meilleur modèle de périodicité. Le modèle qui s'ajuste le mieux au AIC est celui qui présente un cycle annuel. Nous avons évalué la performance générale de la méthode à l'aide de données simulées avec des échantillons de tailles différentes et des temps divers de formation et de durée des paires de bandes de croissance. Les essais de simulation montrent que la performance de la méthode est satisfaisante avec les échantillons de taille moyenne. La méthode devrait améliorer la précision de la détermination d'âge et pourrait s'appliquer à toutes les espèces qui possèdent des paires de bandes de croissance périodique.

[Traduit par la Rédaction]

Introduction

Elasmobranch species (sharks, skates, and rays) are mostly large vertebrates with long life spans, late maturity, and low fecundity, which renders them vulnerable to perturbations imposed by external factors such as fisheries (Musick et al. 2000). Recent reports have shown that shark populations have declined worldwide as commercial fisheries have expanded (Baum et al. 2003; Baum and Myers 2004; Ward and Myers 2005). These reports may have had a substantial influence on several recent conservation decisions. However, some shark scientists commented that their analyses were inadequate and did not capture the complete picture of all shark populations that had been documented (Burgess et al. 2005). Accurate biological information is essential to avoid incorrect quantification of the effects of fisheries on fish populations and for successful management of fisheries.

Information on the ages of fish populations is fundamental to the estimation of biological parameters such as growth rate, mortality rate, and productivity. Erroneous age estimates have resulted in serious errors in the management and understanding of fish populations (Campana 2001; Goldman 2005). For example, the commercial fishery industry associated with the Pacific ocean perch (*Sebastes alutus*) off the west coast of Canada may have lost millions of dollars in profit due to the underestimation of the age of this population (Beamish and McFarlane 1983).

Periodic growth increments formed in calcified structures are commonly used to determine the age of fish. For elasmobranch fish, growth band pairs in the vertebral centra and dorsal fin spines are often used to estimate age and growth. Most authors of reports of the age of elasmobranch populations have assumed that growth increments occur annually, but few have tested this assumption. Population assessment and management are greatly affected by the determination of whether growth band deposition is annual or biannual (Campana et al. 2002; Piercy et al. 2007).

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Table 1. Data for the shortfin mako shark (*Isurus oxyrinchus*) in the North Pacific.

	Month											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Total sample size	31	23	4	12	16	16	22	7	8	45	37	6
No. of opaque bands	24	10	1	3	3	2	3	1	1	25	21	4
Proportion	0.77	0.43	0.25	0.25	0.19	0.13	0.14	0.14	0.13	0.56	0.57	0.67

Note: Number of opaque bands is the number of observations for which the periphery of the centrum was an opaque band. Proportion is the number of opaque bands divided by the total sample size for each month.

Therefore, it is important to elucidate the temporal periodicity of growth band deposition for various species and for populations within species.

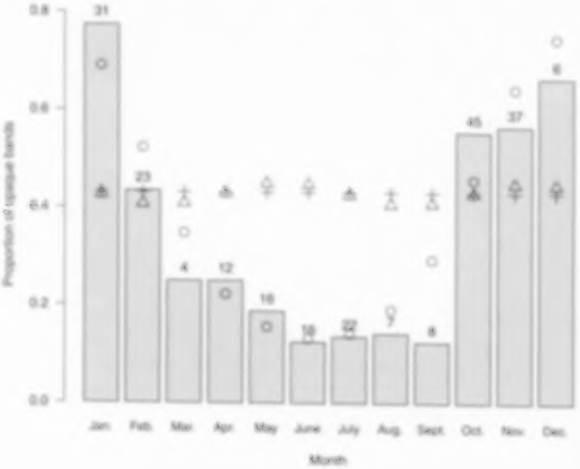
Various approaches exist for validating the age estimation of fish (Cailliet et al. 1986; Cailliet 1990; Campana 2001). Of a variety of methods, the mark–recapture technique for chemically tagged fish commonly has been used for wild fish species. However, because recapture is difficult for many species, centrum edge analysis (CEA) and marginal increment analysis (MIA) are frequently employed for elasmobranchs (Goldman 2005). The underlying principle of CEA and MIA is that the width and (or) density of the outermost increment will exhibit a yearly sinusoidal cycle when plotted against the month of capture if the growth band pairs are formed annually. CEA compares the opacity and translucency (width and (or) density) of the centrum edge, whereas MIA typically compares the width of the ultimate developing band with the width of the last fully formed band pair (Goldman 2005; Cailliet et al. 2006). Although marginal increments are most easily discerned in young fish, extrapolation of such results to older fish may result in errors (Campana 2001). In addition, accurate classification of edge types as opaque or translucent is difficult, which reduces the resolution of the technique and renders interpretation of the results difficult. Despite such problems, these methods are frequently used to study elasmobranch ages because of their modest sample size requirements and low cost (Cailliet et al. 1986, 2006; Cailliet 1990). The results of such studies, particularly those involving CEA, have often been interpreted subjectively as "having the appearance of a cycle". Nevertheless, when combined with additional techniques such as MIA, CEA can provide supportive evidence to validate the periodicity of band pair formation (Cailliet et al. 2006). Rigorous statistical analysis of such results should facilitate their objective and quantitative interpretation (e.g., Cappo et al. 2000).

In this article, we describe a novel statistical method for validating growth increment periodicity using CEA, assuming that discrimination of edge types is accurate. Data on the shortfin mako shark (*Isurus oxyrinchus*) in the North Pacific (Semba et al. 2009) are used to illustrate an application of our method. To examine its performance, we applied the model to simple simulated data sets. Finally, we discuss the merits and problems of our approach.

Materials and methods

Vertebral samples of the shortfin mako were collected by research and commercial vessels from the North Pacific between 1992 and 2005. The centrum was dissected from the vertebra after the connective tissue was removed. The edge of the centrum was categorized as convex or concave using microtopography of the three-dimensional structure on the surface of the centrum by shining reflected light (Semba et al. 2009). The convex and concave structures corresponded to opaque (hypomineralized) and translucent (hypermineralized) growth bands (Semba et al. 2009). We used this conventional terminology in the current study (Cailliet et al. 2006).

Fig. 1. The relative frequency of opaque bands on the periphery according to month for shortfin mako sharks (*Isurus oxyrinchus*) sampled in the North Pacific. The number on each bar denotes the total sample size for the month. Crosses, open circles, and open triangles denote values predicted by models N, A, and B, respectively.



We used the monthly frequency of peripheral opaque bands throughout the year to verify the periodicity of growth band pairs (Table 1). The vertebrae of neonates, which display only birthmarks, were excluded from this analysis because birthmarks differ from normal growth band pairs. The relative monthly frequency of peripheral opaque bands is shown (Fig. 1). Of the 227 samples, 98 had opaque bands on the periphery of the centrum. Although the data presented appear to exhibit an annual cycle, as indicated by a single peak (Fig. 1), monthly sample sizes differed greatly, making it difficult to formulate a conclusion based on objective and quantitative grounds. In addition, as we are unable to determine the probability of a biannual cycle when traditional statistical tests such as analysis of variance (ANOVA) are used, we need to use the following statistical model to facilitate objective and quantitative evaluations of the data.

A new method for validating periodicity

Because the data in Fig. 1 are binary and the sample size differs between months, precision with respect to the proportion of opaque bands also differs between months. Furthermore, the data are periodic. Therefore, we combine a statistical model for binary data with a statistical model for circular data (Fisher 1993).

The assumptions of the model are as follows: (*i*) discrimination between opaque and translucent growth bands is accurate; (*ii*) individual fish have identical and invariant growth band periodicities; (*iii*) the growth bands of each individual in the population are formed at similar times, even if multiple band pairs are formed within a year; and (*iv*) after a growth band pair has formed, the subsequent growth band pair forms within at least 1 year.

The first assumption confirms that the measurement of the edge type is correct without error. From the second assumption, there is no possibility that some fish have an annual cycle and other fish have a biannual cycle. The third assumption ensures that there is at least one peak in the occurrence of growth band pairs. The fourth assumption prohibits slow events such as the biennial production of one growth band pair, even if there is periodicity.

The periodicity of growth band formation is categorized as no cycle, an annual cycle, or a biannual cycle. Although it is easy to extend the model to include more periodicities, such as a triannual cycle, in theory, the model was limited to a biannual cycle at most in light of practical application.

We assume that a vector $\boldsymbol{x} = (x_1, \ldots, x_n)$ is a response variable in which one of the elements x_i equals 1 for an opaque band and 0 for a translucent band. A vector $\boldsymbol{x}$ is linked to a covariate $\boldsymbol{m} = (m_1, \ldots, m_n)$, which is the month in which the *i*th sample was collected. Each $m_i \in \{1, \ldots, 12\}$ is linked to the first day of the month $y(m_i)$, which is expressed in radians. Given this notation and using a binomial model, the probability mass function of the observations $\boldsymbol{x}$ given the parameter θ is

$$(1)\qquad P(\boldsymbol{x}|\theta) = \prod_{i=1}^{n} \left\{ \frac{\gamma}{y(m_i+1) - y(m_i)} \int_{y(m_i)}^{y(m_i+1)} f(u|\mu,\rho)\mathrm{d}u \right\}^{x_i} \cdot \left\{ 1 - \frac{\gamma}{y(m_i+1) - y(m_i)} \int_{y(m_i)}^{y(m_i+1)} f(u|\mu,\rho)\mathrm{d}u \right\}^{1-x_i}$$

where $\theta = (\gamma, \mu, \rho)$, $y(1) = 0$, $y(2) = (31/365)\cdot 2\pi, \ldots, y(13) = 2\pi$, γ is an adjustment parameter to ensure that the probability of the edge type is between 0 and 1, μ is the day (radian) corresponding to the peak of the probability distribution of opaque bands, and ρ is the dispersion parameter in the probability distribution of opaque bands. Note that the model takes differences in monthly sample sizes into consideration. The parameter γ has to satisfy the following constraint:

$$(2)\qquad 0 \le \gamma \le \left[\max\left\{ \frac{1}{y(m_i+1) - y(m_i)} \int_{y(m_i)}^{y(m_i+1)} f(u|\mu,\rho)\mathrm{d}u \right\} \right]^{-1}$$

When a growth band pair is formed annually, the density function $f(u|\mu,\rho)$ is simply a von Mises distribution, which is a common statistical distribution for circular data (Fisher 1993):

$$(3)\qquad f(u|\mu,\rho) = \frac{\exp\{\rho\cos(u-\mu)\}}{2\pi I_0(\rho)}$$

where $0 \le \mu < 2\pi$, $0 \le \rho < \infty$, and

$$(4)\qquad I_0(\rho) = \sum_{i=0}^{\infty} \frac{\rho^{2i}}{2^{2i}(i!)^2}$$

is the modified Bessel function of order zero. Some approximations used to calculate the modified Bessel function are given in Fisher (1993). We used the "CircStats" package in the R statistical software (R Development Core Team 2007).

When a growth band pair is formed biannually, the density function is a finite mixture of von Mises distributions:

$$(5)\qquad f(u|\mu,\rho) = \frac{0.5\exp\{\rho\cos(u-\mu)\}}{2\pi I_0(\rho)} + \frac{0.5\exp\{\rho\cos(u-\mu-\pi)\}}{2\pi I_0(\rho)}$$

where $0 \le \mu < \pi$ and $0 \le \rho < \infty$.

When the growth bands have no cycle, the density function is the same as that given by eq. 3 with $\rho \to \infty$. In this case, eq. 3 converges to the uniform distribution, U[0, 2π] and the number of parameters in eq. 1 is reduced to one, i.e., $\theta = \gamma$. Hereafter, we term the model without a cycle "model N", the model with an annual cycle "model A", and the model with a biannual cycle "model B".

For all cases, the parameter θ is estimated using a maximum likelihood approach by maximizing the logarithm of eq. 1. The numerical optimization was carried out using the Nelder–Mead or the BFGS method in "optim" of the R statistical software (R Development Core Team 2007).

As the aforementioned models are not nested, we must not use a likelihood ratio test to determine whether a growth band pair is formed for no cycle, an annual cycle, or a biannual cycle. Instead of a likelihood ratio test, we used the Akaike information criterion (AIC; Burnham and Anderson 2002). The AIC is defined by multiplying the negative log-likelihood plus the number of estimated parameters by 2. The AIC not only rewards goodness of fit by using the log-likelihood, but also imposes a penalty for overfitting by including the number of estimated parameters. The model with the lowest AIC value is preferred because it is estimated to be closest to the unknown reality that generated the data.

A simple simulation trial

We conducted a simple simulation experiment to evaluate the performance of our model. The simulated data were constituted as follows. (*i*) Determine whether a growth band pair has no cycle, an annual cycle, or a biannual cycle (m = 0 (no cycle), m = 1 (annual cycle), m = 2 (biannual cycle)). (*ii*) The total number of fish is set to K. (*iii*) The median formation time t_i of the first band for each fish i has a truncated normal distribution $\mathrm{TN}(t_i \mid c\mu, (cw_1/d)^2)$ with a mean of μ, a standard deviation of cw_1/d, and $-2cw_1/d \le t_i - c\mu \le 2cw_1/d$, where $c = 2\pi/365$ is a factor that converts days to radians, d is 1 for m = 0 or 1 and 2 for m = 2, and μ is a randomly generated value between 1 January and 31 December. When m = 0, μ is assigned separately to each individual. When m = 1 or 2, μ is common to all individuals. (*iv*) When a growth band pair is formed biannually, the median formation time u_i of the subsequent band has a uniform distribution $\mathrm{U}(t_i - 0.8\pi, t_i + 1.2\pi)$, given the formation time of the former band t_i and assuming periodicity of a half year on average. (*v*) The duration of each band is modeled by adding a uniform distribution with mean cw_2/d and width of ±20%, $\mathrm{U}(0.8cw_2/d, 1.2cw_2/d)$, to both sides of the median band formation date t_i, and u_i if m = 2. (*vi*) The sample size of fish for each month has a negative binomial distribution $\mathrm{NB}(n, k)$ with mean n and variance $n + n^2/k$, and the date of the month sampled has a uniform distribution. (*vii*) The presence or absence of an opaque band and the corresponding month are recorded for individual fish randomly sampled according to the monthly sample size set in (*vi*). (*viii*) The three models with no, annual, and biannual cycles (i.e., models N, A, and B) are fitted separately to the data generated in (*vii*) and the AICs are calculated. (*ix*) Repeat steps *ii* to *viii* R times and then record the number of times that the AIC selected the wrong model.

The parameters K = 10 000, R = 1000, and k = 2 were fixed. w_1 is related to the variation of the formation timing for the opaque band and determines how the opaque band occurs within a year. For example, when m = 1, μ = 1 August, and w_1 = 90, the formation timing of opaque band for each individual fluctuates, and the probabilistic distribution of the median formation period is the truncated bell curve with a center of 1 August and a standard deviation of 90 days. w_2 controls the duration of the opaque band after it is formed. For example, when m = 1, t_i = 1 August and w_2 = 90, the opaque band for individual i continues to be, on average, from 90 days before 1 August to 90 days after 1 August. As w_1 and w_2 are the most influential control parameters in our simulation, we performed the simulation for w_1 = 80, 90, and 100 and w_2 = 80, 90, and 100 with 10 intervals, respectively (shown in Table 2). The mean monthly sample size was changed over n = 5, 10, 20, 30, 40, and 50 (Table 2). The dispersion parameter of the negative binomial distribution k was selected because the corresponding value of the shortfin mako in the North Pacific was 2.3. As k decreases, the variation in monthly sample size increases and its influence on the results tends to become large. As a performance statistic, we used the error rate, which is the ratio of the number of times that the AIC selected the wrong model to the number of iterations R. The error rates were termed types N, A, and B for the true models with no cycle, an annual cycle, and a biannual cycle, respectively.

Results

Three models were fitted to data from shortfin makos collected in the North Pacific (Fig. 1). The observed histogram has a peak between December and January. Because sample sizes differed between months, precision also differed between months. Of the three models, the annual cycle model (indicated by circles in Fig. 1) best fitted the data. The AICs were 312.44 for model N, 271.89 for model A, and 316.24 for model B. This large difference in AIC between the models strongly supports an annual edge cycle in shortfin makos of the North Pacific. The peak for model A corresponded to 21 December.

Examples of simulations for a mean monthly sample size of 20 are provided (Figs. 2–4). As w_1 and w_2 increased, it became increasingly difficult to determine the number of peaks visually. For example, when the true model was model A with an annual cycle of growth band pairs, one peak in spring and another in autumn were evident for w_1 = w_2 = 100 (Fig. 3*i*). As a result, when using only visual inspection, one could erroneously judge from this data set that the growth band pairs had a biannual cycle, although actually there was an annual cycle.

The results of the simulation trials are summarized (Table 2). When the true model was model N, i.e., no cycle on a growth band pair, type-N error rates were 20% or more for various patterns of w_1 and w_2. That is to say, even if there was no cycle on a growth band pair, the AIC wrongly selected models with an annual or biannual cycle for growth band pairs with a probability of 20%. When the true model was model A, the error rates were resilient to changes in w_1, whereas they were sensitive to changes in w_2. Type-A error rates were less than 15% for moderate monthly sample size, i.e., 20. When the true model was model B, type-B error rates were resilient to changes in w_2, whereas they were sensitive to changes in w_1. Although type-B error rates tended to exceed type-A error rates, they were still relatively small and generally less than 20% for monthly sample sizes of 20.

Discussion

Mixture models generally characterize data sets well and have broad applications (e.g., Francis and Campana 2004). As the usual regularity conditions are not appropriate to analysis of mixture models, likelihood ratio tests and information criteria cannot be used formally to assess the number of components (Mooney et al. 2003). However, our model has a constrained mixture distribution with fixed mixing proportions, and eq. 3 is not nested by eq. 5. In addition, the range of the μ parameter differs between eqs. 3 and 5. Hence, the problem of identifiability of parameters does not

Table 2. Error rates from the simulation.

		$w_1 = 80$			$w_1 = 90$			$w_1 = 100$		
w_2	n	NC	AC	BC	NC	AC	BC	NC	AC	BC
80	5	0.255	0.188	0.316	0.252	0.161	0.509	0.238	0.185	0.643
	10	0.244	0.055	0.142	0.249	0.056	0.253	0.250	0.067	0.458
	20	0.209	0.012	0.073	0.243	0.025	0.095	0.221	0.045	0.204
	30	0.234	0.017	0.075	0.226	0.020	0.090	0.260	0.038	0.131
	40	0.214	0.017	0.090	0.226	0.024	0.071	0.230	0.030	0.104
	50	0.194	0.014	0.077	0.232	0.031	0.069	0.212	0.025	0.104
90	5	0.252	0.346	0.299	0.230	0.340	0.461	0.232	0.370	0.617
	10	0.231	0.132	0.124	0.228	0.149	0.239	0.243	0.172	0.421
	20	0.245	0.041	0.061	0.265	0.047	0.086	0.222	0.070	0.224
	30	0.231	0.025	0.057	0.211	0.028	0.066	0.229	0.051	0.108
	40	0.238	0.013	0.064	0.229	0.028	0.058	0.213	0.049	0.068
	50	0.212	0.015	0.064	0.229	0.028	0.067	0.228	0.048	0.050
100	5	0.240	0.542	0.338	0.264	0.558	0.483	0.263	0.559	0.628
	10	0.222	0.303	0.104	0.243	0.307	0.258	0.247	0.339	0.439
	20	0.225	0.136	0.043	0.217	0.128	0.078	0.220	0.143	0.205
	30	0.239	0.053	0.039	0.222	0.057	0.058	0.214	0.073	0.113
	40	0.217	0.036	0.044	0.255	0.044	0.048	0.226	0.064	0.077
	50	0.228	0.038	0.043	0.233	0.041	0.046	0.230	0.056	0.057

Note: Error rate is the ratio of the number of times that the AIC selected the wrong model to the total number of iterations in the simulation; w_1 and w_2 are the variation of formation timing and the duration for the opaque band set in the simulation, respectively; n denotes the monthly mean sample size; NC, AC, and BC denote that the true periodicity is no cycle, an annual cycle, or a biannual cycle, respectively.

apply to our model when there is a biannual cycle of growth band pairs. The use of information criteria in our model is therefore justified in terms of the usual regularity conditions, whereas the likelihood ratio test is not used because of the absence of a nested structure.

Type-N error rates were about 20% for various cases. The AIC tends to overestimate the number of components, whereas the Bayesian information criterion (BIC) selects a more parsimonious model. When we used BIC instead of AIC, type-N error rates were close to zero, even when the sample size was small. On the other hand, type-A and type-B error rates were large, even for large sample sizes. Burnham and Anderson (2002) reported that BIC performed poorly and that they preferred AIC to BIC. We used AIC because of their recommendation. Using AIC, type-A and type-B error rates were acceptably small with sample sizes of 20 or 30 when w_1 and w_2 did not exceed 100. The type-N error rate of about 20% seems relatively large in comparison with the 5% significance level used for usual statistical tests. However, AIC is algebraically equivalent to a likelihood ratio test of size 15.7% in the special case of testing for inclusion of a single extra term (Buckland et al. 2001). In addition, the 20% error rate compares favorably with other AIC assessments (Richards 2005).

We selected the best model even when the differences among the AIC values were slight. However, Burnham and Anderson (2002) suggested that because of unavoidable sampling error, the model with the lowest AIC value is not necessarily the best Kullback–Leibler model and an AIC difference greater than 2 is necessary to identify definitively a model as the best model in terms of the Kullback–Leibler distance. In our North Pacific shortfin mako example, the annual cycle model had an AIC difference of more than 10, which suggests strongly that this was the best model (Burnham and Anderson 2002).

The AIC enabled us to estimate probabilistic and quantitative plausibility between various periodicities in a growth band pair. The ANOVA and Kruskal–Wallis tests can only be used to determine whether or not there is periodicity; they cannot be used to determine which annual or biannual periodicity is quantitatively more plausible when periodicity is present. With our model, we could use the Akaike weights to evaluate differences between weights as evidence (Burnham and Anderson 2002). Quantitative assessment of such evidence enables risk analysis to be used for fishery management (Hilborn and Walters 1992; Francis and Shotton 1997).

The model is applicable to data sets that lack data for some months. When we applied our model to the data set of leopard sharks (*Triakis semifasciata*) given in table 1 of Smith (1984), which lacked observations for April, July, and August, the resultant AICs were 27.02 for model N, 24.15 for model A, and 31.02 for model B. As the sample size was small (n = 25), the differences among the AICs were also small. Nonetheless, our model positively supported annual band formation, which is consistent with the results of Smith (1984), who employed a mark–recapture technique with the bone-labeling reagent oxytetracycline.

In the current simulation trials, we did not completely assess possible effects of violation of the model assumptions. The first assumption mentioned above, accurate discrimination between opaque and translucent zones, is particularly important as the identification of edge types is influenced by many factors and is not necessarily straightforward (Cailliet et al. 2006). Because the main focus of our method is to determine the periodicity statistically rather than to estimate accurate parameter values, the method is expected to be robust against any small violation of the first assumption, i.e., a few erroneous discriminations of opaque and translucent zones. However, serious measurement errors will produce

Fig. 2. Example plots of simulation results with a sample size of 20 and a true number of modes equal to 0. The proportion denotes the relative frequency of opaque bands for each month. w_1 and w_2 are the variation of formation timing and the duration for the opaque band set in the simulation, respectively: (*a*) $(w_1, w_2) = (80, 80)$; (*b*) $(w_1, w_2) = (80, 90)$; (*c*) $(w_1, w_2) = (80, 100)$; (*d*) $(w_1, w_2) = (90, 80)$; (*e*) $(w_1, w_2) = (90, 90)$; (*f*) $(w_1, w_2) = (90, 100)$; (*g*) $(w_1, w_2) = (100, 80)$; (*h*) $(w_1, w_2) = (100, 90)$; (*i*) $(w_1, w_2) = (100, 100)$.

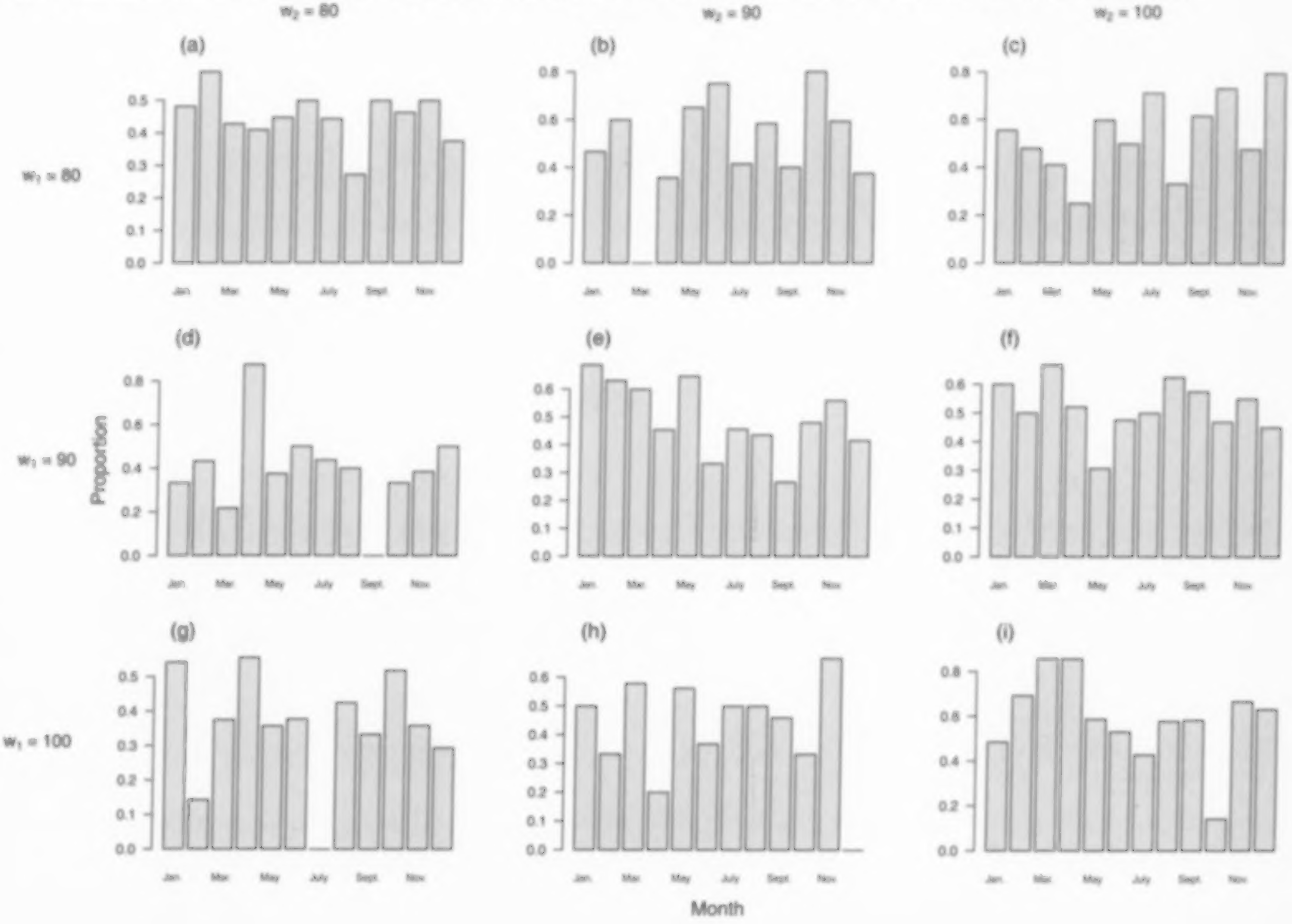


Fig. 3. Example plots of simulation results with a sample size of 20 and a true number of modes equal to 1. The proportion denotes the relative frequency of opaque bands for each month. w_1 and w_2 are the variation of formation timing and the duration for the opaque band set in the simulation, respectively: (*a*) $(w_1, w_2) = (80, 80)$; (*b*) $(w_1, w_2) = (80, 90)$; (*c*) $(w_1, w_2) = (80, 100)$; (*d*) $(w_1, w_2) = (90, 80)$; (*e*) $(w_1, w_2) = (90, 90)$; (*f*) $(w_1, w_2) = (90, 100)$; (*g*) $(w_1, w_2) = (100, 80)$; (*h*) $(w_1, w_2) = (100, 90)$; (*i*) $(w_1, w_2) = (100, 100)$.

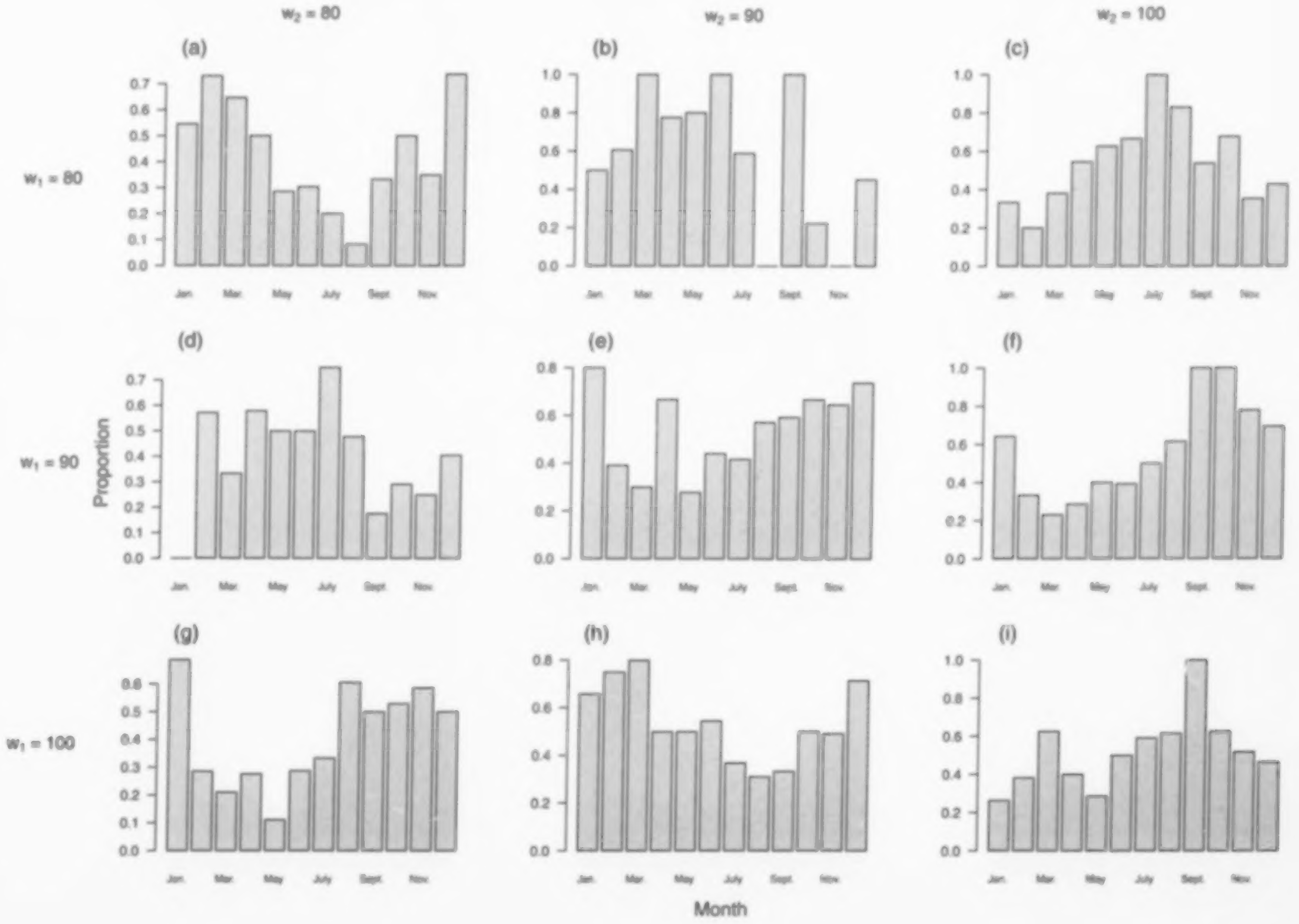
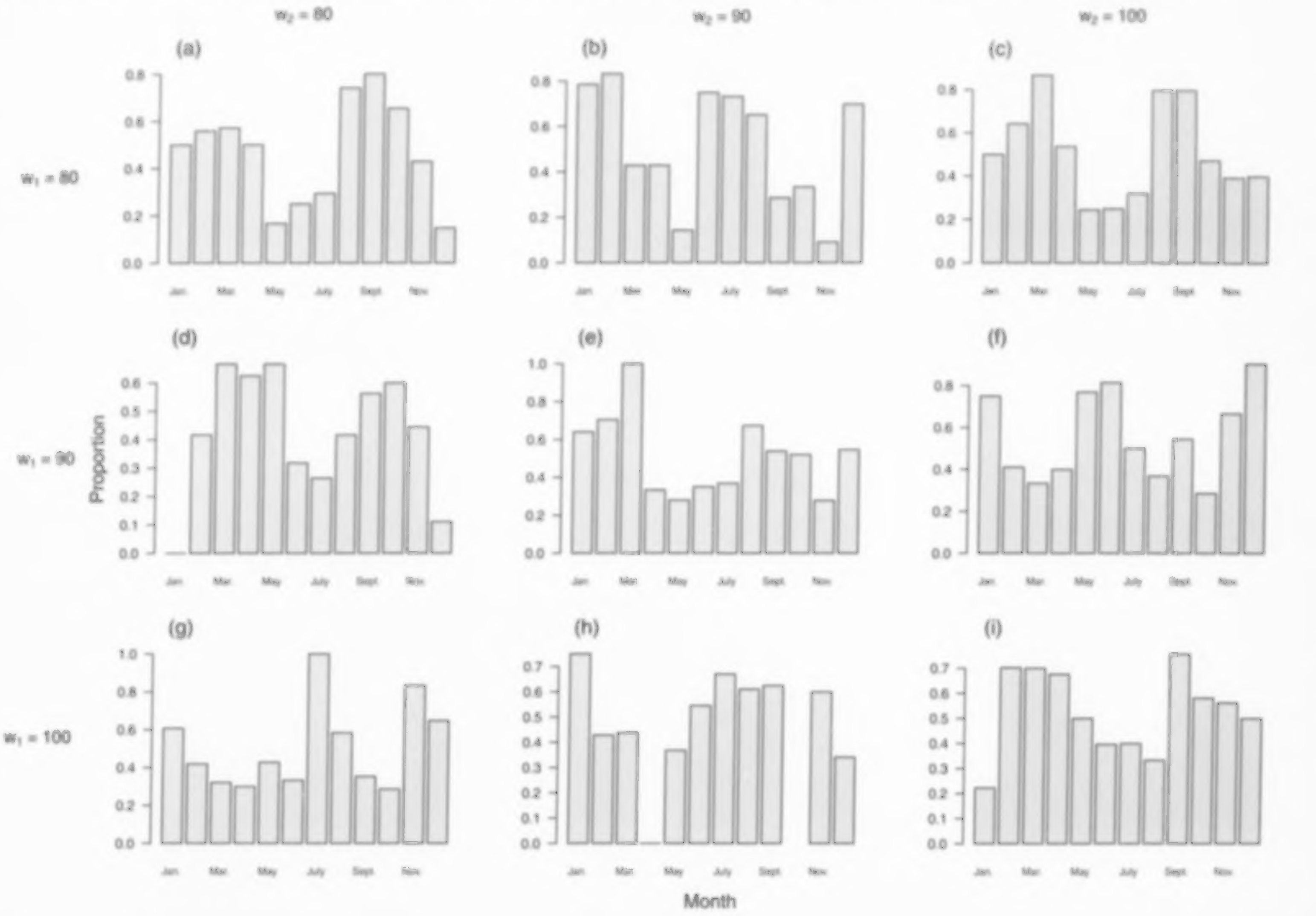


Fig. 4. Example plots of simulation results with a sample size of 20 and a true number of modes equal to 2. The proportion denotes the relative frequency of opaque bands for each month. w_1 and w_2 are the variation of formation timing and the duration for the opaque band set in the simulation, respectively: (*a*) $(w_1, w_2) = (80, 80)$; (*b*) $(w_1, w_2) = (80, 90)$; (*c*) $(w_1, w_2) = (80, 100)$; (*d*) $(w_1, w_2) = (90, 80)$; (*e*) $(w_1, w_2) = (90, 90)$; (*f*) $(w_1, w_2) = (90, 100)$; (*g*) $(w_1, w_2) = (100, 80)$; (*h*) $(w_1, w_2) = (100, 90)$; (*i*) $(w_1, w_2) = (100, 100)$.



worse results. A simulation with erroneous discrimination would provide us with the information on the maximum permissible amount of errors. Another solution is to put the estimation of measurement errors in discrimination into the model using the data discriminated by the independent researchers with equivalent abilities.

As marginal increments are most easily discerned in young fish, results obtained with young fish are often extrapolated to older fish (Campana 2001). However, older fish do not necessarily have the same ageing structure as young fish. If possible, data for young fish and older fish should be separated, even if such analysis may necessitate a large sample size.

To examine the sensitivity of the basic probabilistic model, we carried out additional simulation tests using a wrapped Cauchy distribution (Fisher 1993; Morales et al. 2004), which is an alternative to the von Mises distribution. The wrapped Cauchy distribution provided similar results to those obtained using the von Mises distribution.

Our simulation trials showed that type-B error rates increased as w_1 increased, whereas type-A error rates increased as w_2 increased. When w_1 increased, the formation time of opaque bands extended over a long period of time, bringing the frequency of occurrence for opaque bands close to a uniform distribution and making the peak unclear. Therefore, an increase in w_1 tends to induce underestimation of the number of peaks. When w_2 increased, the duration time of opaque bands extended over a long period, with the result that opaque bands were apt to occur in any month and the peak was uneven. Consequently, for a large value of w_2, an annual cycle may tend to be mistaken for a biannual (or shorter) cycle.

When $w_1 = 100$, provided that the true number of modes is one, an opaque band has to be formed throughout the year with an extremely high probability. When $w_2 = 100$, opaque bands have long duration times of more than half in a cycle. This may be unrealistic if growth bands are produced depending on environmental factors that fluctuate seasonally (Goldman 2005). In fact, an opaque band was considered to be deposited during about half of a year in some studies (e.g., Smith 1984; Hale and Lowe 2008). In addition, when $w_1 = 100$ or $w_2 = 100$, the number of peaks becomes unclear. Therefore, we consider our simulation trials to have included the worst-case scenario. Even in the worst case, our model performs relatively well for moderate sample sizes (20 or 30, on average, for each month).

The mean monthly sample size for the shortfin mako in the North Pacific is about 20, and the simulation trials showed relatively good performance for this sample size. The histogram for North Pacific shortfin mako has one clear peak at first sight. The shortfin mako migrates in temperate waters of variable temperature (Casey and Kohler 1992), so there is less probablity that it stays in the same environment for over a half year. Therefore, $w_1 = 100$ and (or) $w_2 = 100$ in the simulations may be too large for the shortfin mako, and the shortfin mako result should be more decisive with a low error rate. These results, and the considerable difference in AICs, strongly indicate that the growth band pairs of the shortfin mako in the North Pacific have an annual cycle.

Pratt and Casey (1983) assumed that growth band pairs of the North Atlantic shortfin mako have a biannual cycle. However, Campana et al. (2002) and Ardizzone et al. (2006) suggested that an annual cycle was present based on preliminary bomb radiocarbon methods. Similarly, for the scalloped hammerhead shark (*Sphyrna lewini*) in the Pacific Ocean, Chen et al. (1990) and Tolentino and Mendoza (2001) reported a biannual cycle of growth band pair formation, whereas Piercy et al. (2007), who used MIA, suggested an annual cycle for the same species in the Atlantic Ocean. Such uncertainty concerning age determination may have serious economic consequences for fisheries, as well as a great ecological impact on fish stocks (Beamish and McFarlane 1983; Campana 2001). In particular, the practice of visually assessing periodicity, as is common for CEA, can produce misleading results. Since the report of Beamish and McFarlane (1983), considerable progress has been made in age validation and verification. Methods involving direct validation of age using mark–recapture techniques are particularly valuable. Nonetheless, an objective comparison of direct results obtained using mark–recapture methods with indirect results obtained using approaches such as CEA and MIA would facilitate validation of the periodicity of growth band pair formation. Our method performed well with a relatively low error rate, even when visual assessment was difficult. Our approach is the first strictly statistical method for age verification of elasmobranchs using CEA and, in principle, could be applied to all fish species that have periodic growth band pairs. The approach presented here should greatly improve the accuracy of age determination in elasmobranchs.

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Reproductive behavior and relative reproductive success of natural- and hatchery-origin Hood Canal summer chum salmon (*Oncorhynchus keta*)

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Abstract: Estimates of the relative fitness of hatchery- and natural-origin salmon can help determine the value of hatchery stocks in contributing to recovery efforts. This study compared the adult to fry reproductive success of natural-origin summer chum salmon (*Oncorhynchus keta*) with that of first- to third-generation hatchery-origin salmon in an experiment that included four replicate breeding groups. Hatchery- and natural-origin chum salmon exhibited similar reproductive success. Hatchery- and natural-origin males obtained similar access to nesting females, and females of both types exhibited similar breeding behaviors and durations. Male body size was positively correlated with access to nesting females and reproductive success. The estimates of relative reproductive success (hatchery/natural = 0.83) in this study were similar to those in other studies of other anadromous salmonids in which the hatchery population was founded from the local natural population and much higher than those in studies that evaluated the lifetime relative reproductive success of nonlocal hatchery populations.

Résumé : Des estimations de la fitness relative des saumons d'origine naturelle et de pisciculture peuvent aider à déterminer la valeur que représentent les stocks de pisciculture dans les efforts de récupération. Notre étude compare le succès reproductif de l'adulte à l'alevin chez des saumons kéta (*Oncorhynchus keta*) d'été d'origine naturelle à celui de saumons de pisciculture de première à troisième génération dans une expérience qui comporte deux séries de quatre groupes reproducteurs. Les saumons kéta d'origine naturelle et de pisciculture ont des succès reproductifs semblables. Les mâles de pisciculture et les mâles d'origine naturelle ont des accès semblables aux femelles au nid et les femelles des deux types ont des comportements reproducteurs et des durées de fraie semblables. Il y a une corrélation positive entre la taille corporelle du mâle et son accès aux femelles en nidification et son succès reproductif. Les estimations du succès reproductif relatif (pisciculture/origine naturelle = 0,83) dans notre étude sont semblables à celles d'autres études d'autres saumons anadromes dans lesquelles la population de pisciculture a été fondée à partir de la population naturelle locale et elles sont beaucoup plus élevées que celles d'études qui ont évalué le succès reproductif au cours de la vie de populations de pisciculture d'origine non locale.

[Traduit par la Rédaction]

Introduction

Breeding populations of Pacific salmon (*Oncorhynchus* spp.) and steelhead (*Oncorhynchus mykiss*) typically include fish of hatchery and natural origin. Natural population productivity, which is a critical parameter for determining status under the United States Endangered Species Act (McElhany et al. 2000), can be difficult to estimate in populations that include hatchery-produced salmon because the relative fitness of hatchery fish is highly variable, usually unknown, and typically less than that of co-mingling natural populations (Berejikian and Ford 2004; Araki et al. 2008). Estimates of the relative fitness of hatchery- and natural-origin salmon can provide data to parameterize productivity models, improve the assessments of natural population productivity (e.g., McClure et al. 2003), and assist in determining the value of hatchery stocks in contributing to recovery efforts (Fraser 2008).

Current information on relative fitness of hatchery- and natural-origin salmon (i.e., ratio of hatchery recruits per spawner to natural recruits per spawner) is biased towards one iteroparous species (steelhead) and towards nonlocally adapted hatchery populations that have been highly domesticated (sometimes intentionally; reviewed by Berejikian and Ford (2004) and Araki et al. (2008)). However, Araki et al. (2007*a*) measured relative lifetime fitness of hatchery- and

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natural-origin steelhead and concluded rapid fitness loss (one to two generations) that had a genetic basis. In contrast, McGinnity et al. (2004) indicated no difference in relative genetic fitness (embryo to adult) in a locally derived, multigenerational hatchery-reared Atlantic salmon (*Salmo salar*) population. Hatchery-reared steelhead and other species (Atlantic salmon, brown trout (*Salmo trutta*), and coho salmon (*Oncorhynchus kisutch*)) for which relative fitness studies have been published (reviewed by Berejikian and Ford (2004) and Araki et al. (2008)) typically spend between one-third to one-half of their lifetime in the hatchery prior to release, so environmental influences of hatchery rearing on the reproductive success of those species may be substantial (Fleming et al. 1997).

Summer chum salmon (*Oncorhynchus keta*) are semelparous, typically live to 3 or 4 years of age, and tend to spawn in the lower reaches of streams in late summer and their offspring migrate to sea upon emergence from late winter to early spring (Quinn 2005). Summer chum salmon populations in Hood Canal, Washington, USA, underwent severe declines during the 1970s and 1980s and were extirpated from some streams (Washington Department of Fish and Wildlife and Point No Point Treaty Tribes (WDFW–PNPTC) 2000). A supplementation program involving the collection and artificial spawning of locally derived, natural-origin adults was initiated in the Quilcene River in 1992 and ended with the last fish being released in 2002. The Hood Canal summer chum salmon evolutionarily significant unit was listed as threatened under the US Endangered Species Act in 1999. Hatchery-produced summer chum salmon continue to be released into some Hood Canal streams. Information on the relative reproductive success (RRS) of hatchery- and natural-origin salmon provides an important measure of the efficacy of this strategy in contributing to overall recovery efforts for this species.

We tested the null hypothesis that the relative adult-to-fry reproductive success of hatchery-origin summer chum salmon does not differ from that of natural-origin fish by quantifying the ability to produce offspring in a spawning channel near Big Beef Creek (Kitsap County, Washington). This study did not directly test genetic fitness because hatchery- and natural-origin fish resided in different environments during their first month of life. However, it compares hatchery and natural fish that experienced the same environment (seawater) for a much longer portion of the life cycle (over 90%) than previous studies on other species (reviewed by Araki et al. (2008)). We compared reproductive behavior between hatchery- and natural-origin chum salmon to understand whether any behavioral differences would be associated with potential differences in reproductive success. Chinook salmon hatchery programs can influence age- and size-at-maturity (Hankin et al. 1993; Knudsen et al. 2006), and the same may occur for other species. Therefore, we also investigated relationships between body size, behavior, and reproductive success and discuss implications for salmon hatchery programs designed to supplement natural populations. By evaluating reproductive success to the juvenile offspring life stage, this study isolates differences between hatchery and natural fish that may occur during the freshwater phase of the life cycle (breeding, incubation, and outmigration) and does not address relative fitness after seawater entry.

Materials and methods

Study populations and experimental design

The US Fish and Wildlife Service's Quilcene National Fish Hatchery has collected summer chum salmon adults in the Quilcene River estuary for supplementation programs since 1992. Prior to that, no summer chum salmon hatchery programs were present in the Quilcene River or anywhere else in Hood Canal. The adult chum salmon were spawned artificially and their offspring were reared to approximately 1 g (1.5 months) and released into the Quilcene River. All released hatchery fish had their adipose fins removed. Those fish returning in 2004 and 2005 that did not have an adipose fin came from the hatchery program and those with an adipose fin were naturally produced either from natural-origin fish or from hatchery-reared fish spawning naturally (as early as 1995). Thus, we do not know the ancestry of the hatchery- and natural-origin fish used in this study prior to their parental generation. However, the hatchery fish used in the experiments had had at least one more generation of hatchery ancestry (i.e., their parents were spawned in the hatchery) than the natural-origin fish used in the study, the parents of which spawned naturally.

Adult chum salmon were collected by beach seining in Quilcene Bay on 7 and 20 September 2004 and 7 and 21 September 2005. On each date, seining continued until the required number of males and females from each population was obtained. Immediately after each collection, the fish were transported by tank truck to the University of Washington's Big Beef Creek Research Hatchery in oxygenated transport containers. Upon arrival at Big Beef Creek, the fish were weighed, measured, and marked with individually numbered Peterson disc tags (Floy Tag Co., Seattle, Washington). Tissue samples for DNA genotyping were collected and preserved in 100% nondenatured alcohol. In each year, fish collected on the first date were released together into one side of a quasinatural spawning channel (described below), and fish collected on the second date were stocked into the other side of the channel. The stocking date, origin (hatchery or natural), body size, and age of the fish used in both years of the experiment are provided (Table 1).

The study was conducted in an experimental stream channel at the Big Beef Creek facility. The portion of the stream channel used in this study measured 35 m long × 4 m wide and was supplied with water (approximately 0.05 $m^3 \cdot s^{-1}$) from a combination of Big Beef Creek and a local groundwater well. The channel was divided lengthwise into two similar sections (east channel and west channel) prohibiting movement of adults between them. The stream channel had substrate that ranged from silt and sand to gravel 20 cm in diameter. A mix of natural early succession vegetation and mature cedar trees comprised the riparian vegetation, which is typical of spawning habitat for Hood Canal summer chum salmon. Observation blinds were placed alongside both sides of the channel to minimize disturbance of the fish during observations.

Behavioral observations

In 2004, behavioral observations were conducted three times each day at approximately 0700, 1300, and 1900 h. In 2005, observations were conducted twice daily, once in the

Table 1. The number (*n*), mean length (± standard deviation, SD), mean weight (±SD), and mean age (±SD) of adult hatchery- and natural-origin (natural) chum salmon (*Oncorhynchus keta*) stocked into east and west sides of the spawning channel at Big Beef Creek in 2004 and 2005.

		2004				2005			
		n	Length (mm)	Weight (g)	Age (years)	*n*	Length (mm)	Weight (g)	Age (years)
Females									
East	Hatchery	12	680 (27)	3708 (522)	3.9 (0.3)	10	643 (44)	3060 (748)	3.2 (0.4)
	Natural	12	682 (23)	3729 (415)	4.0 (0.0)	14	647 (29)	3188 (470)	3.5 (0.6)
West	Hatchery	12	683 (23)	3770 (425)	3.5 (0.5)	10	638 (27)	3030 (441)	3.4 (0.7)
	Natural	12	681 (28)	3697 (490)	3.9 (0.3)	12	638 (43)	2971 (660)	3.3 (0.6)
Males									
East	Hatchery	12	681 (27)	3708 (522)	3.9 (0.3)	10	643 (44)	3060 (748)	3.2 (0.4)
	Natural	12	682 (23)	3729 (415)	4.0 (0.0)	14	647 (29)	3188 (470)	3.3 (0.7)
West	Hatchery	12	684 (24)	3770 (425)	3.5 (0.5)	10	637 (27)	3030 (441)	3.7 (0.5)
	Natural	12	681 (28)	3697 (490)	3.9 (0.3)	12	638 (43)	2971 (660)	3.8 (1.0)

Note: The sex ratios are similar to those used in the majority of other reproductive success studies (reviewed by Araki et al. 2008).

morning and once in the evening. Observers began at a randomly selected location of the stream channel and recorded several behaviors for each female (Table 2). Typically, each female was observed for up to 10 min; females that were inactive, stationary, and not near other females were observed for a shorter period of time (5 min) before the next female was sampled. All behaviors observed during that time were recorded.

Selection on female spawning ground entry timing has been demonstrated in studies of other Pacific salmon (Dickerson et al. 2002; Ford et al. 2006). Early spawners may have access to high-quality nest sites but risk nest superimposition by later-spawning females (van den Berghe and Gross (1989). Because hatchery- and natural-origin salmon can differ in spawn timing, we calculated the following durations from the observed behaviors: (*i*) latency to onset of sexual activity (time from introduction to the first signs of nest digging), (*ii*) duration of sexual activity (time from first to last episodes of nest digging), and (*iii*) duration of nest guarding (time from first to last observation of nest guarding). We compared these traits between the hatchery- and natural-origin females.

In both years, we quantified how often individual males dominated access to a sexually active female (dominant male) and how often they held a "satellite" position downstream of a courting pair. We identified all males associated with a nest-building female. The male that maintained closest proximity to a nesting female, delivered a greater number of attacks towards other males than it received, and exhibited courtship behaviors was considered dominant (Berejikian et al. 2001). When aggressive contests appeared to be equal and proximity to the female was similar, we used coloration patterns to identify the dominant fish based on our own observations and the descriptions provided by Schroder (1981). In male chum salmon, the "bar" color pattern consists of black and red streaks that run perpendicular to the length of the fish, overlying a mottled green–yellow background that becomes tan on the dorsal surface of the peduncle. The "stripe" pattern, which mimics female coloration, consists of a single black–purple band that runs parallel to the length of the fish. Bar patterns were present in clearly dominant fish and striped patterns were present in clearly subdominant fish.

The time of death for all fish was recorded. The numbers of eggs retained in the body cavities of females were counted.

Fry collection

The downstream end of the experimental spawning channel was fitted with wood-framed, wire mesh traps in attempts to collect all fry that emerged from the gravel and emigrated. The traps were checked each morning during the emergence and outmigration period, and all fry were counted. We subsampled the fry populations such that emigrants proportionately represented each day's collection over the entire outmigration period (Fig. 1). With these methods, we assumed that outmigrating fry were sampled in an unbiased manner and results of the pedigree analyses reflect adult-to-outmigrant reproductive success. In a previous study, sampling 5.5% of emergent fry was sufficient to represent offspring of 98% of naturally spawning steelhead trout (Berejikian et al. 2005). In the present study, collections were sampled at an average (± standard deviation, SD) daily rate of 7.3% (±1.2%) in 2005 and 8.8% (±4.2%) in 2006 for days in which more than 10 fry were captured. However, on days when fewer than 10 fry were captured, which were typically at the very beginning and end of the outmigration season, one fry was preserved. It was important to sample fry at the same rate each year to produce comparable data sets. However, the total number of outmigrant juveniles in 2006 was substantially less than in 2005, which resulted in unequal numbers of offspring available for genotyping (see Results).

Genotyping and pedigree analysis

All of the adult summer chum salmon stocked into the experimental stream channels in 2004 and 2005 were uniquely identified by analyzing genomic DNA isolated from fin tissue samples. Genomic DNA was subjected to polymerase chain reactions (PCR) to amplify eight loci: *One18* (Scribner et al. 1996), *One102*, *One106*, *One111* (Olsen et al. 2000), *Ots3* (Banks et al. 1999), *Ots103* (Small et al. 1998),

Table 2. Definitions of behaviors to determine the status of individual female chum salmon (*Oncorhynchus keta*) over the course of the experiments in 2004 and 2005 (cf. Fleming et al. 1997; Schroder et al. 2008).

Behavior	Definition
Inactive	Not yet showing signs of sexual activity; not attended by male(s)
Courted	Female is paired with 1 or more males, but no spawning behaviors are being displayed; record all males associated with female
Test digging	Scattered digging not concentrated in one area
Nest digging	Digging that results in deepening and shaping of a nest depression
Spawning	Female deposits eggs while accompanied by an ejaculating male
Egg covering	Female quickly covers eggs with very frequent, 2–3 body flexure digging motions immediately after releasing eggs
Redd covering	Prolonged digging around perimeter of redd (8–12 body flexures) with body almost parallel to substrate that results in gravel being mounded on top of the redd
Nest guarding	Holding over redd and exhibiting aggression toward other fish
Senescent	Female has spawned, looks thin, exhibits no sexual activity, and is usually away from her redd

OtsG311, and *OtsG422* (Williamson et al. 2002). A fragment analysis was conducted on the PCR products using a genetic analyzer (model 3100; Applied Biosystems, Foster City, California). Results of the electrophoretic runs were analyzed using Genescan and Genotyper software (Applied Biosystems) to determine the genotypes of every individual for each locus.

A pedigree analysis was conducted to determine the adult-to-fry reproductive success of individual adult summer chum released into the spawning channel. The subsampled outmigrant juveniles were genotyped for the same loci as the adults. Parentage assignments were made using the computer program CERVUS (Marshall et al. 1998) by initially using an exclusionary approach. If exclusion alone was insufficient to identify a single parental pair for a particular juvenile, a likelihood method was used to assign parentage.

Statistical analyses

We tested the hypothesis that reproductive behavior and adult-to-fry reproductive success of hatchery and natural chum salmon did not differ over the course of this 2-year study. For females, we tested for differences in prespawning, spawning, and nest-guarding durations, egg retention, number of matings, and reproductive success. Males were tested for differences in dominance and satellite frequencies and reproductive success. Data were analyzed by a two-way mixed-model analysis of variance (ANOVA). Origin (hatchery or natural) was the fixed main effect of interest and breeding group, representing all spawners in one channel in one year (i.e., 2004 east (E), 2004 west (W), 2005E, and 2005W) was the random factor. The following variables were square-root-transformed to improve normality: number of fry produced (males and females), number of mates (males and females), and spawning duration (females only). The relative number of fry produced by each adult chum salmon was the primary variable of interest. Therefore, we also calculated the RRS of hatchery fish by dividing the mean number of fry produced per hatchery fish by the mean number produced per natural fish for each breeding group. A value of 1.0 would reflect equal reproductive success. One RRS value was produced for each breeding group, and we presented the mean ± 95% confidence interval for the estimates of RRS ($N = 4$). This provided a basis for comparison with studies on other species (Berejikian and Ford 2004; Araki et al. 2008). Using the same paired-sample approach, we conducted a post-hoc power analysis to determine the number of independent breeding groups that would be required to detect the calculated mean differences in reproductive success at $\alpha = 0.05$ and $\beta = 0.80$.

We tested for assortative mating using separate 2 × 2 contingency table analyses, with a Yates correction, for each breeding group. Male parent (hatchery or natural origin) and female parent (hatchery or natural origin) were the column and row categories, respectively. Matings were evident by the production of at least one offspring as determined by the pedigree analysis. Assortative mating could lead to reproductive isolation and divergence between hatchery and natural populations and may reflect mate choice related to unobserved differences in phenotypic characters between hatchery- and natural-origin spawners. Nonassortative mating would suggest similar mate choice and intrasexual selection patterns. We tested for correlations between male and female body weight and the various behavioral and reproductive success response variables by simple linear regression analyses. Separate analyses were conducted within each breeding group.

Results

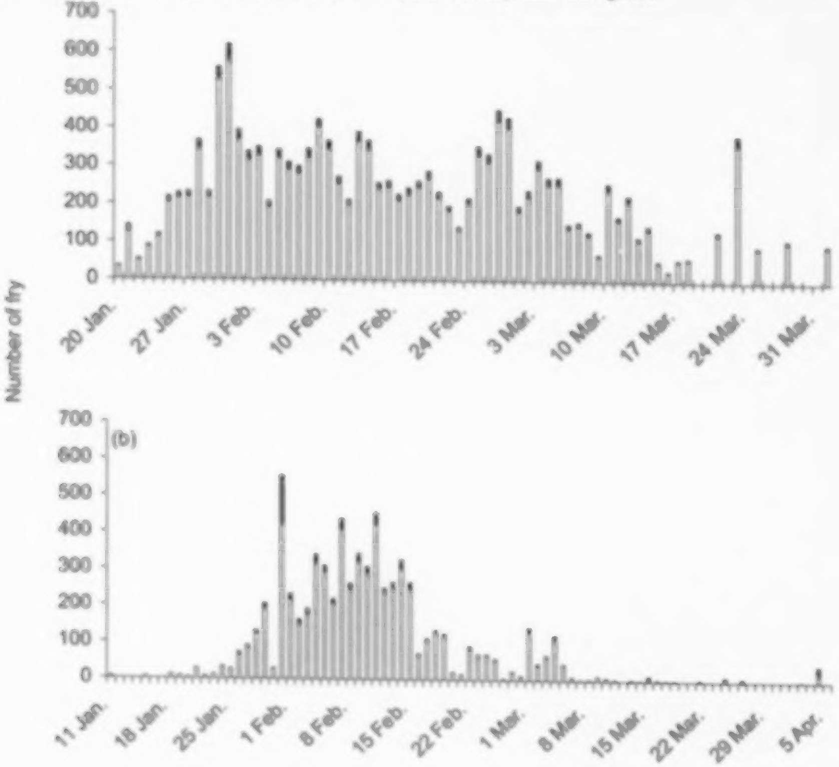
Pedigree analyses

A total of 1208 of the 1230 fry sampled in 2005 (2004 spawning season) were assigned to a single pair mating. Of those, 97% were assigned by exclusion and 3% by likelihood analysis ($P > 0.95$). A total of 457 of 487 fry sampled in 2006 (2005 spawning season) were assigned to a single pair mating. Of those, 83% were assigned by exclusion and 17% by likelihood analysis ($P > 0.95$). Twenty-two fry were dropped from the 2005 data set and 30 fry were dropped from the 2006 data set because they could not be assigned to two parents with a high level of confidence.

Female behavior and reproductive success

None of the statistical interactions between stock origin (natural and hatchery) and breeding group (2004E, 2004W, 2005E, and 2005W) was significant for any of the female behaviors, matings, or reproductive success measures ($P > 0.18$ in all cases), with the exception of spawning duration ($P = 0.05$). In 2005W, hatchery females took longer on aver-

Fig. 1. Outmigrating summer chum (*Oncorhynchus keta*) fry captured in (*a*) 2005 and (*b*) 2006. Shaded portions of the bars represent the number of fry subsampled for the DNA pedigree analyses. Fry were caught in traps at the downstream end of the spawning areas. The channel was electrofished on 23 and 25 March and 1 April 2005 (all data shown) and on 4 April 2006 (22 fry caught; data not shown). In each year, all fry migrating from the two sides of the channel were able to mix and were captured in a single trap. Emigrating fry averaged less than 40 mm and 0.4 g in each year, suggesting that they outmigrated shortly after emergence.



age to complete spawning than the natural-origin females, whereas spawning durations in the other three groups were similar. The overall adult-to-fry reproductive success of hatchery females was not significantly different from that of natural-origin females ($P = 0.27$; Fig. 2). The RRS point estimates were less than 1.0 in three of the breeding groups (0.62 in 2004E, 0.77 in 2004W, and 0.15 in 2005E) and greater than 1.0 in the other (1.34 in 2005W). The results of a post-hoc power analysis ($\alpha = 0.05$, $\beta = 0.80$) suggests that 10 replicate breeding groups with the same number of females per breeding group used in this study would have been required to detect the estimated 28% reduction in reproductive success (Fig. 2).

The mean number of mates per female (hatchery origin = 1.7; natural origin = 1.9) was similar ($P = 0.47$). Hatchery- and natural-origin females did not differ significantly in time to onset of spawning or spawning duration (Fig. 3). Natural-origin females were observed nest guarding on average 40 h longer than hatchery females, but this difference was also not significant ($P = 0.18$; Fig. 3). Finally, female hatchery- and natural-origin fish retained similar numbers of eggs at death ($P = 0.58$). Reproductive success, egg retention, and each of the behavioral response variables differed significantly among breeding groups ($P < 0.05$).

Male behavior and reproductive success

None of the statistical interactions between stock origin and spawner group was significant for any of the male behaviors or reproductive success measures ($P > 0.17$ in all cases). The overall adult-to-fry reproductive success of hatchery-origin males was very similar to that of natural-origin males ($P = 0.53$; Fig. 2). As with the females, the RRS estimates were less than 1.0 in 2004E (0.81), 2004W (0.64), and 2005E (0.95) and greater than 1.0 in 2005W (1.70). The results of a post-hoc power analysis ($\alpha = 0.05$, $\beta = 0.80$) suggest that 24 replicate breeding groups with the same number of males per breeding group used in this study would have been required to detect the 3% estimated increase in reproductive success exhibited by hatchery-origin fish (Fig. 2).

The mean number of mates per hatchery-origin male (1.4) equaled that for natural-origin males ($P = 0.98$). Hatchery- and natural-origin males were observed with similar frequency in the dominant ($P = 0.99$), satellite 1 ($P = 0.61$), and satellite 2 ($P = 0.21$) positions in male hierarchies and thus appeared equally capable of obtaining access to nesting females. Reproductive success and the frequency of attending females as either a dominant or satellite male differed significantly among the four breeding groups ($P < 0.05$).

Fig. 2. The relative reproductive success of hatchery-produced and natural-origin summer chum salmon (*Oncorhynchus keta*). A value of 1 would represent equal reproductive success of hatchery- and natural-origin fish. The bars represent mean values (±95% confidence intervals around the mean value; $N = 4$ breeding groups).

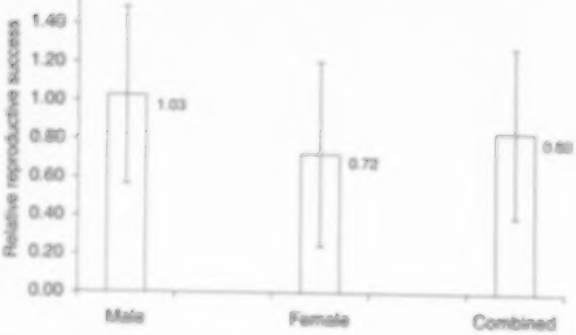
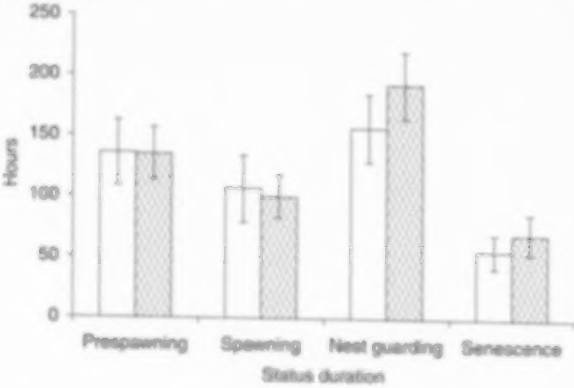


Fig. 3. Graph of mean spawning durations (±95% confidence interval) for each behavioral status quantified for hatchery-origin (open bars) and natural-origin (stippled bars) female chum salmon (*Oncorhynchus keta*).



Body size and dominance

We assumed that dominance and reproductive success may each depend on body size, but not the reverse; therefore, we first tested for a positive correlation between body size (independent variable) and dominance frequency (dependent variable). Male body weight was positively correlated with dominance frequency in 2004W, 2005E, and 2005W but not in 2004E (Fig. 4). Male body weight was significantly and positively correlated with reproductive success in 2004E, 2004W, and 2005E but not in 2005W (Fig. 4). The standardized residuals of the weight vs. dominance frequency regression relationship were positively and significantly correlated with reproductive success in 2004E ($r^2 = 0.185$, $P = 0.010$) and 2005W ($r^2 = 0.121$, $P = 0.036$) but not in 2004W ($r^2 = 0.001$, $P = 0.921$) or 2005E ($r^2 = 0.071$, $P = 0.092$). Thus, the potential effect of dominance on reproductive success is not entirely an advantage conferred by large body size. Female body size was not significantly correlated with any of the female reproductive behaviors, egg retention, number of mates, or reproductive success in any of the four breeding groups ($P > 0.05$ for all tests).

Mating combinations

The summer chum salmon in this study did not appear to mate assortatively with respect to origin (hatchery or natural) in any of the four breeding groups (2004W and 2004E, $\chi^2 = 0.00$, $P = 1.00$; 2005W, $\chi^2 = 0.483$, $P = 0.487$; 2005E, $\chi^2 = 0.671$, $P = 0.413$; overall, $\chi^2 = 0.540$, $P = 0.463$). Of the 161 matings detected in the pedigree analysis for all four breeding groups combined, 33 were hatchery × hatchery, 42 were natural origin × natural origin, 46 were hatchery female × natural-origin male, and 42 were natural-origin female × hatchery-origin male.

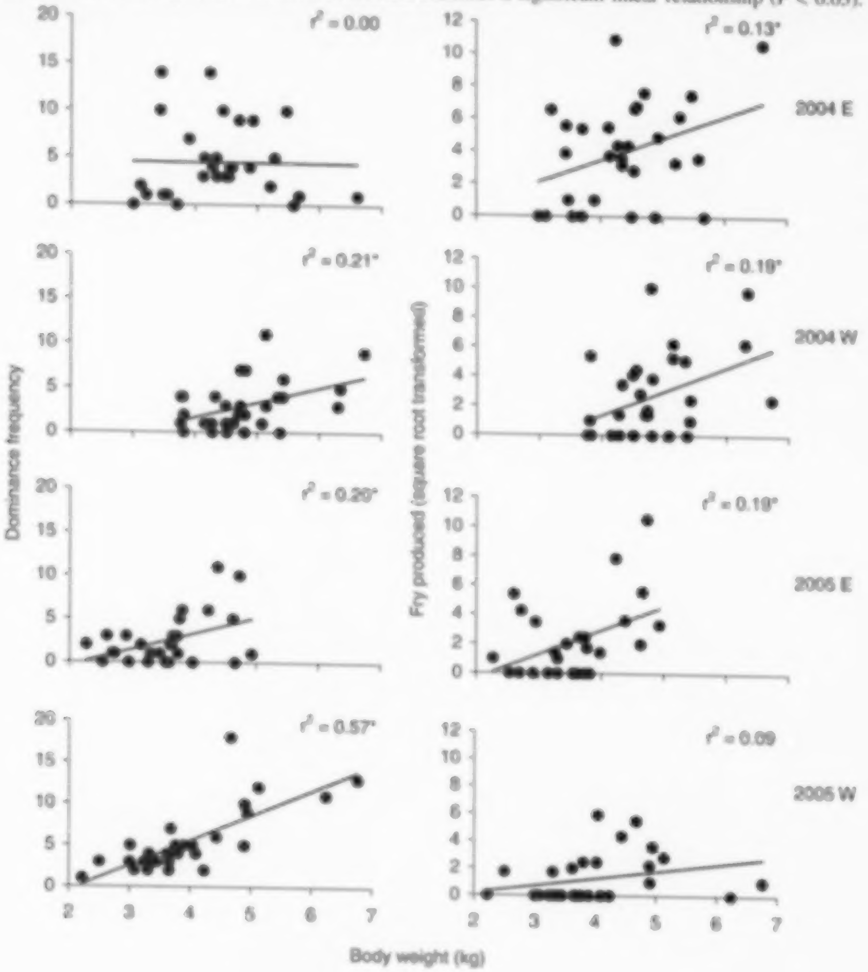
Discussion

We found no significant differences in reproductive success of hatchery- and natural-origin summer chum salmon. Mean RRS estimates for both males and females have fairly large 95% confidence intervals, which indicate low statistical power to detect differences between populations. Although variation in individual reproductive success was high, the overall mean reproductive success of hatchery- and natural-origin males was nearly identical. Therefore, our results suggest no real between-population differences in male reproductive success. The RRS of hatchery females averaged 0.72 and may indicate reduced fitness that we were unable to detect. However, hatchery- and natural-origin males and females exhibited similar reproductive behavior, spawned synchronously, and did not mate assortatively with respect to origin, providing no indication of reduced breeding performance of hatchery fish that might compromise offspring production.

With one exception, the few studies that have evaluated the RRS of hatchery fish derived from the local wild broodstock have indicated low to moderate reductions in relative fitness. Ford et al. (2006) estimates of the relative lifetime fitness of hatchery coho salmon in Minter Creek, Washington (0.74 for females and 1.01 for males), approximated the adult-to-fry estimates in our study, although their estimates of adult-to-fry fitness were somewhat higher (females = 1.16, males = 1.26). Araki et al. (2007*b*) found nearly equal relative fitness in hatchery- and natural-origin winter steelhead; however, their updated analysis in 2007, including a total of six run years, indicated mean RRS of 0.88 and 0.70 for females and males, respectively. Schroder et al. (2008) did not calculate relative fitness in the same way in which we and others have; they estimated equal egg deposition and a 5.6% reduction in the egg-to-fry survival of female spring Chinook salmon (*Oncorhynchus tshawytscha*) spawning with the natural founder population in an experimental stream channel. In stark contrast, several studies of steelhead have demonstrated dramatically reduced reproductive success of nonlocal hatchery fish (e.g., Leider et al. 1990; Kostow 2004; Araki et al. 2007*b*). Similar studies of nonlocal farmed Atlantic salmon (Fleming et al. 2000) and nonlocal wild Atlantic salmon (McGinnity et al. 2004) have also indicated comparably poor relative fitness.

One interpretation of the apparent performance disparity between locally derived versus nonlocal hatchery populations is that utilizing local-origin adults for broodstock

Fig. 4. Relationships between male body weight (kg) and courting frequency (left column) and fry production (right column, square-root-transformed). An asterisk next to the squared correlation coefficient indicates a significant linear relationship ($P < 0.05$).



might ameliorate reductions in hatchery fish fitness; however, one recent study would predict a different outcome. Araki et al. (2007*a*) estimated that hatchery-origin fish with two hatchery-origin parents had only 55% of the relative lifetime RRS of hatchery-origin fish with one hatchery-origin and one natural-origin parent, suggesting a genetic basis for the fitness loss. A follow-up modeling exercise suggested that either simultaneous selection on multiple traits or intense selection coupled with very high heritability could generate fitness loss of this magnitude (Araki et al. 2008). Our study suggests reproductive similarities between natural-origin summer chum salmon and hatchery-produced salmon. Our study primarily tested potential domestication selection following one to three generations of artificial spawning, hatchery incubation, and brief juvenile rearing in addition to the environmental effects of early experience in the hatchery. The results of ongoing studies on Chinook salmon and steelhead throughout the Pacific Northwest US will soon provide a further context and help in assessing whether the findings of Araki et al. (2008) reflect (*i*) a species-specific vulnerability to rapid fitness loss, (*ii*) some artifact of the particular river system, population, or hatchery practices, or (*iii*) a more widespread phenomenon in hatchery-produced salmonids.

Domestication selection (adaptation to the hatchery environment) may result from prespawning captivity in the hatchery collection facility, broodstock selection, and artificial spawning (Campton 2005; McLean et al. 2005), adaptation to incubation and early rearing environments (reviewed by Fraser 2008), and selection on hatchery-induced phenotypic variation in the postrelease environment (Reisenbichler et al. 2004). The present study is the first to examine a species with a very brief juvenile freshwater life-history phase. Juvenile chum salmon begin migration to sea within days or weeks after emergence, so the opportunity for domestication selection on juvenile traits combined with juvenile hatchery rearing experience that might affect reproductive success is more limited than for other salmon. Therefore, our results probably pertain more to genetic effects of artificial spawning and incubation, because the hatchery- and natural-origin chum salmon experienced similar environments through nearly their entire postemergence lifetime. All previous studies of hatchery salmon RRS have involved species that spend a year (coho salmon and stream-type Chinook salmon) or more (steelhead and Atlantic salmon) in fresh water and therefore are subject to selection pressures in the hatchery-rearing environment for an extended period, are probably more prone to unnatural selection on hatchery-modified phenotypes (Kostow 2004) after release, and are more strongly affected by early experience (Fleming et al. 1997).

All RRS studies of hatchery- and natural-origin salmon using DNA pedigree analyses include some degree of competition, because the hatchery- and natural-origin fish were spawning in the same river or spawning channel (Berejikian and Ford 2004). We chose high spawner densities (approximately one female and one male per 3 m^2 of available habitat) to provide a competitive environment and to determine whether competitive asymmetries might exist and manifest in differences in breeding success between hatchery- and natural-origin chum salmon. Fleming and Gross (1993) experimentally manipulated spawner densities and found that hatchery coho salmon suffered increasingly lower relative breeding success as spawner densities were increased from 5.0 m^2 to 2.5 m^2 to 1.25 m^2 of habitat per male or female spawner, and the effects were greater on hatchery males than females. The breeding success of farmed Atlantic salmon males decreased as spawner density increased from 3.9 m^2 to 3.6 m^2 to 2.6 m^2 of habitat per male (Fleming et al. 1997) in a similar study. Although the comparisons are across species (coho salmon) and across genera (Atlantic salmon), the densities that we created should have been sufficient to detect competitive asymmetries had they existed.

Hatchery rearing can reduce body size either by advancing age-at-sexual maturity through broodstock selection (Hankin et al. 1993) or through environmentally mediated effects on growth (Larsen et al. 2004; Knudsen et al. 2006). Hatchery fish may also be substantially smaller than natural-origin fish during the first year of returns in new supplementation programs when the hatchery fish are younger, or they may be larger during the last year of a supplementation program when only the oldest hatchery fish return to spawn. Therefore, it is reasonable to predict that hatchery-origin males will exhibit differential breeding success based, in part, on size-mediated access to nesting females. The positive correlations between male body size and breeding success in this study appear to be fairly widespread among anadromous salmonids (Atlantic salmon, Fleming et al. 1997; steelhead, Seamons et al. 2004; coho salmon, Fleming and Gross 1992; pink salmon (*Oncorhynchus gorbuscha*), Dickerson et al. 2002; chum salmon, Schroder 1981; sockeye salmon (*Oncorhynchus nerka*), Quinn and Foote 1994). However, in natural streams, predation, arrival timing, or other factors may present additional or opposing selection factors (Dickerson et al. 2005; Seamons et al. 2007).

The Hood Canal summer chum salmon conservation program (WDFW–PNPTC 2000) should reasonably expect that the reproductive success of hatchery-origin fish is sufficient to contribute to rebuilding of depleted stocks along with other management actions, including harvest reductions and habitat improvement projects. To guide artificial propagation of salmon more broadly, it will be important to have several measures of relative fitness for each anadromous salmonid species that is bred in captivity and released with the explicit goal of contributing to natural reproduction.

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Contrasting patterns of resource utilization between juvenile estuarine predators: the influence of relative prey size and foraging ability on the ontogeny of piscivory

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Abstract: In aquatic systems, ontogenetic patterns of resource utilization strongly influence growth and survival, particularly during early life stages. We compared prey resource use and evaluated potential factors affecting the timing of the shift to piscivorous feeding in two juvenile estuarine fish predators: striped bass (*Morone saxatilis*) and bluefish (*Pomatomus saltatrix*). In New York Bight estuaries, bluefish shift to piscivory earlier in ontogeny and consume larger relative prey fish sizes compared with striped bass. Predator gape allometries are similar and did not determine maximum prey sizes eaten. Experimental results revealed marked differences in foraging abilities between predators, with bluefish realizing much greater foraging efficiency compared with striped bass feeding on identical fish prey. Both predators demonstrated lower feeding efficiency and grew relatively poorly when feeding on invertebrates compared with fish prey. When held together under limited prey conditions, bluefish exploited a greater proportion of available prey at the expense of striped bass. Our findings highlight the importance of the availability of appropriately sized forage fishes to the ontogeny of piscivory and provide evidence that predator–prey size relationships and disparate foraging abilities can generate inter- and intra-specific variation in patterns of resource utilization and predator growth.

Résumé : Dans les systèmes aquatiques, les patrons ontogéniques d'utilisation des ressources influencent fortement la croissance et la survie, particulièrement durant les premiers stades de la vie. Nous comparons l'utilisation des ressources en proies et évaluons les facteurs potentiels qui affectent le moment du changement vers un régime alimentaire piscivore chez deux jeunes poissons prédateurs estuariens, le bar rayé (*Morone saxatilis*) et le tassergal (*Pomatomus saltatrix*). Dans les estuaires du golfe de New York, les tassergals deviennent piscivores plus tôt dans leur ontogénie et consomment des proies de taille relativement plus grande que les bars rayés. Les allométries de l'ouverture de la bouche sont semblables et ne déterminent pas la taille maximale de proie consommée. Des données expérimentales indiquent des différences marquées dans l'habileté de recherche de nourriture entre ces prédateurs et les tassergals atteignent une efficacité de recherche de nourriture beaucoup plus grande que celle des bars rayés qui se nourrissent des mêmes poissons proies. Lorsqu'ils s'alimentent d'invertébrés plutôt que de poissons, les deux prédateurs montrent une efficacité alimentaire réduite et croissent relativement mal. Gardés ensemble en présence de proies limitées, les tassergals exploitent une proportion plus élevée des proies disponibles au détriment des bars rayés. Nos résultats soulignent l'importance de la disponibilité de poissons fourrage de taille adéquate dans l'ontogénie de l'ichtyophagie et démontrent que les relations de taille entre les prédateurs et les proies, ainsi que les différences d'habileté de recherche de nourriture, peuvent produire des variations inter- et intraspécifiques dans les patrons d'utilisation des ressources et la croissance des prédateurs.

[Traduit par la Rédaction]

Introduction

Ontogenetic niche shifts maximize growth and survival and are thought to be adaptive (Werner and Gilliam 1984). The diet shift from invertebrate to fish prey by piscivorous fishes is known to be an important transitional stage that results in markedly increased growth rates for many species (Juanes and Conover 1994; Olson 1996; Galarowicz and Wahl 2005). Predators that shift to fish prey early in ontogeny benefit from larger body size at a young age, which can provide a refuge from size-dependent sources of mortality (e.g., predation and overwinter starvation; Sogard 1997; Hurst 2007). Because of advantages in growth and survival conferred during early life, the timing of the onset of piscivory has been implicated as a major determinant of year

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class strength for freshwater piscivores (Buijse and Houthuijzen 1992; Olson 1996). Given the likely strong selective pressures to shift to a fish diet, what are the factors that affect the timing of the onset of piscivory? Timing of spawning, water temperature, predator morphology, growth rates during the invertebrate feeding stage, and predator and prey population dynamics have all been shown to influence the shift to piscivory (e.g., Buijse and Houthuijzen 1992; Amundsen et al. 2003; Galarowicz et al. 2006). These factors may often operate synergistically to generate community patterns of prey fish density and predator–prey size relationships, which contribute principally to the encounter and capture probabilities that appear most critical in determining the ability of a predator to forage on fish prey (Juanes et al. 2002).

In a multispecies review of the ontogeny of piscivory, Mittelbach and Persson (1998) noted that size-specific information on diets and growth rates was often lacking for many freshwater piscivores, which made detecting general interspecific patterns difficult. Among several predator species, the authors did identify traits such as size at hatch and gape size that were broadly related to the timing of the shift to piscivory. However, they did not find differences in the sizes of prey fishes consumed by different piscivore species, which they attributed partly to the shortage of more refined measures (e.g., size-dependent capture success and attack rates) of piscivore foraging ability. They concluded that to determine the extent of variation in the onset of piscivory among species, as well as determine the principle factors responsible for this variation, would require more focused studies that combined field comparisons with laboratory experimentation. Given that the timing of the shift to piscivory has been found to contribute largely to the eventual success of piscivore cohorts (e.g., Buijse and Houthuijzen 1992; Olson 1996), there is a clear need for comparative ontogenetic information to more fully understand the environmental, behavioral, and morphological causes of inter- and intra-specific variation.

Striped bass (*Morone saxatilis*) and bluefish (*Pomatomus saltatrix*) are predatory fishes found in western North Atlantic estuarine and continental shelf waters (Bigelow and Schroeder 1953; Kendall and Walford 1979). Striped bass are anadromous and spawn in several river systems on the US east coast (e.g., the Hudson River, several Chesapeake Bay rivers, and the Roanoke River – Albemarle Sound system). Juvenile striped bass from the Hudson River population occupy several New York Bight estuaries and occur predominantly in these systems during their second summer of life (i.e., at age-1). Juvenile bluefish also occupy New York Bight estuaries during their first summer of life (i.e., at age-0). Following spawning by adults in oceanic waters, juvenile age-0 bluefish from a spring spawning event recruit to estuaries in June, while those from an early to mid-summer spawning event recruit in August–September (McBride and Conover 1991). Along the US Atlantic coast, the adult stages of both species are primarily piscivorous in their feeding habits (Hartman and Brandt 1995*a*; Walter et al. 2003). However, diet studies of juvenile bluefish and striped bass in New York Bight estuarine environments provide evidence for an early and abrupt shift to piscivory by age-0 bluefish (Juanes and Conover 1995) and a later and gradual shift to piscivory by age-0 and age-1 striped bass (Gardinier and Hoff 1982; Buckel and McKown 2002; Jordan et al. 2003). Since a large body of ecological evidence suggests the likelihood of strong selective pressures for an early onset of piscivory (see above), why do striped bass exhibit a delayed onset of piscivory compared with the early shift to piscivorous feeding by sympatric bluefish? Prey availability alone would not seem to explain the interspecific differences, since the fish prey that are available to bluefish in New York Bight estuaries should also be available to striped bass.

In this study, the following questions are addressed: Are there consistent differences in the timing of the onset of piscivory between bluefish and striped bass in estuarine environments of the New York Bight? Might differences in predator morphology or foraging ability contribute to diverse patterns in the use of fish prey by these predators? And finally, do differences in foraging ability result in differential predator growth in a mixed-species environment with limited fish prey? To address these questions, we combined new experimental evidence with previously published field data on feeding habits and a re-examination of behavioral evidence from published laboratory experiments. Specifically, we examined accumulated field data on the diets and prey sizes eaten by juvenile age-1 striped bass and age-0 bluefish in New York Bight estuaries to generate a synthetic view of the timing of the shift to piscivory in these two species. We compared predator gape allometries to assess whether morphological limitations may be important in determining prey sizes eaten. To evaluate interspecific differences in predator foraging ability and related behavioral mechanisms while feeding on common prey, we compared predator capture success and handling times, prey profitabilities, and predator attack behavior based on a re-examination of data from previously conducted laboratory experiments. To confirm differences in predator growth due to prey quality observed previously for juvenile bluefish, we conducted a new experiment to determine growth rates for striped bass when fed unlimited rations of fish versus invertebrate prey. Finally, we completed an additional experiment that tested for the existence of differential foraging abilities in a mixed-predator assemblage through an examination of growth rates realized in a limited prey environment.

Materials and methods

Diet, prey size, and predator morphology

A synopsis of juvenile bluefish and striped bass diets in New York Bight (USA) estuaries was developed from fish collected in the lower Hudson River (41°00′N, 73°54′W) (1974: Gardinier and Hoff 1982; 1990–1993: Buckel et al. 1999) and western Long Island bays (40°40′N, 73°45′W) (1997–1998: Buckel and McKown 2002) during summer and fall of several years. For each study, the percentage of prey by stomach (Gardinier and Hoff 1982) or frequency of occurrence (% FO, all other studies) were standardized to 100% by month and percent invertebrate and fish prey calculated. These values were used to determine a monthly mean for both bluefish and striped bass.

Prey size data for two prey fish species (Atlantic silverside (*Menidia menidia*) and bay anchovy (*Anchoa mitchilli*)) common to the diets of both predators were examined from bluefish collected in the lower Hudson River during 1990–1993 (Scharf et al. 1997) and Great South Bay, New York (40°10′N, 73°10′W) during 1989 (Juanes and Conover 1995), as well as from bluefish and striped bass collected in western Long Island bays during 1997–1998 (Buckel and McKown 2002). Quantile regression analysis was used to determine the upper bounds (maximum prey size) of bivariate relationships between both bay anchovy and Atlantic silverside total length (TL) and predator TL (Scharf et al. 1998). To determine if the upper bounds for each prey type differed between bluefish and striped bass, we applied an ad hoc test statistic based on the standard errors of the quantile regression coefficients that at least asymptotically approaches an *F* distribution (Scharf et al. 1998).

Prey body depths were estimated from regressions relating bay anchovy and Atlantic silverside TL to body depth (Scharf et al. 1997). Striped bass gape height was measured as the distance between the anterior-most points of the lower and upper jaws with the mouth completely open, and throat width was measured as the distance between the cleithrum bones when stretched open to their maximum width. Bluefish gape height and throat width were determined previously from similar measurements and reported in Juanes and Conover (1995) and Scharf et al. (2002). Gape allometries between species were compared using analysis of covariance (ANCOVA).

Laboratory experiments

Size-dependent foraging ability and behavior

The results of single-species experiments to quantify size-dependent foraging ability of bluefish and striped bass have been reported previously in Scharf et al. (2002 and 2003), which evaluated the influence of prey-specific traits (e.g., size, morphology, and behavior) on their vulnerability to predation. Here, we re-examined the results of several of these experiments to evaluate the relative foraging ability of bluefish and striped bass when feeding on common prey. Details of fish capture, transport, laboratory acclimation, and maintenance are carefully outlined in Scharf et al. (2002, 2003). The design and execution of the laboratory experiments that generated some of the data re-examined in this study are also described in a previous publication (text in Methods and tables 1 and 2 in Scharf et al. 2003). All experiments were completed at the NOAA Fisheries James J. Howard Marine Sciences Laboratory at Sandy Hook, New Jersey, USA. We completed additional single-species foraging experiments with striped bass predators feeding on sand shrimp (*Crangon septemspinosa*) between 10 and 35 mm TL that were captured along sandy beaches using 1 m beam trawls and fed pieces of previously frozen fish during laboratory holding. The results of the foraging experiments using striped bass predators and sand shrimp prey have not been reported previously.

Relationships between predator capture success and relative prey size (prey length/predator length ratio) were compared between bluefish and striped bass when feeding on each prey species using ANCOVA. Mean handling times as a function of relative prey size (linearized using $\log_e$ transformation) were compared similarly. Profitability, an index of size-dependent predator foraging efficiency, was also compared qualitatively between predators feeding on each prey type as a function of relative prey size. Profitability was calculated as prey-specific energetic content ($kJ \cdot g^{-1}$) × relative prey mass (prey mass (g) per predator mass (g)) × % capture success × mean handling time^{-1}. Because tank volumes were moderate (475 L) and water clarity was high during single-species experiments, we assumed that encounter probability was close to one at all times, and therefore energetic costs for searching activities were low and independent of prey type or size for both predators. Data for bluefish foraging on sand shrimp presented in Juanes et al. (2001) were compared with experimental results for striped bass foraging on sand shrimp obtained during this study.

Predator foraging behavior was characterized from the video-recorded observations made during the laboratory feeding experiments. Predator schooling behavior, movement patterns within the experimental arena, prey inspection behavior, and attack behaviors were assessed qualitatively. Predator attack velocities as a function of body size were quantified for a random sample of attacks based on distance traveled and the number of video frames (~0.03 $s \cdot frame^{-1}$) elapsed during the attack. Velocities were only measured for individuals moving in a plane orthogonal to the camera. Traveled distances were measured using a grid system positioned within each aquarium and were adjusted for the distance of individuals from the front of aquaria. Least squares regressions were generated to determine the relationship between predator attack velocity and body size for each predator species and were compared using ANCOVA.

Predator growth on shrimp versus fish prey

A 20-day growth experiment was conducted in 2001 to test for an effect of prey type on age-1 striped bass growth. Striped bass were collected in New York Bight estuaries using beach seines. All fish were immediately transported to the Howard Laboratory and acclimated in round tanks (1.8 m diameter × 0.6 m deep) supplied with flow-through seawater from Sandy Hook Bay. Fish were maintained at ambient temperatures (19–22 °C), salinities (23–27 ppt), and photoperiod (14 h light:10 h dark) during the experimental period.

The effect of diet on striped bass growth was tested using two diet treatments that consisted of shrimp (sand shrimp) or fish (Atlantic silverside) prey. Each treatment was replicated three times. Eighteen striped bass were measured (TL; ±1 mm), weighed (±0.01 g), and placed in groups of three into one of six 565 L circular aquaria (1.2 m diameter × 0.5 m depth) supplied with flow-through seawater. Aquaria were assigned randomly to either shrimp- or fish-fed treatments. Live shrimp and fish prey of similar size (prey/predator TL ratios = 0.15–0.30) were provided in unlimited quantities throughout the experimental period, and measurements of striped bass (TL (mm) and weight (g)) were made at the start (day 0), midpoint (day 10), and end (day 20) of the experiment. Dead, uneaten prey were removed and weighed, and temperature and salinity were measured daily.

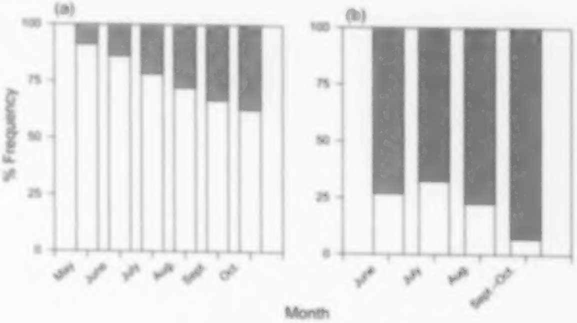
Striped bass growth between shrimp- versus fish-fed treatments was compared using a repeated-measures analysis of variance (ANOVA) with weight as the dependent variable and day as the independent variable. Results for striped bass growth were compared with previously published data for bluefish growth when fed fish versus shrimp diets (Buckel et al. 2004).

Foraging ability in a mixed-predator assemblage

A 50-day experiment was conducted to test for differences in foraging ability among predator species in a mixed-predator assemblage. Bluefish and striped bass, independent from predators used in foraging behavior experiments, were collected, transported, and acclimated to laboratory conditions using methods identical to those used for prior experiments. Fish were measured (TL; ± 1 mm), weighed (±0.01 g), and assigned (see below) to nine circular, experimental mesocosms (2360 L; 2.4 m diameter × 0.6 m depth) supplied with flow-through seawater at ambient conditions. Three experimental treatments, each replicated three times, were employed: six bluefish (single-species), six striped bass (single-species), and three bluefish + three striped bass (mixed-species). Individual predators were assigned randomly to either single- or mixed-species treatments, and measurements (TL (mm) and weight (g)) were made every 10 days. In maintaining equal fish densities across treatments, the experiment employed a substitutive design to measure the relative foraging ability of bluefish versus striped bass (Fausch 1998; Weber and Fausch 2003). For our purposes, the use of a substitutive design eliminated any effect of an overall increase in density and allowed us to measure whether the effect on one species of adding a separate species was distinct from the effect of simply adding more conspecifics. If growth rates in a mixed-predator assemblage differ from expected patterns based on the observations from single-species treatments, one can infer differences in foraging ability (Weber and Fausch 2003).

Prey fish were provided in limited quantities to detect differences in growth rate between mixed- versus single-species treatments if diverse foraging abilities existed in a mixed-predator assemblage. Rather than feed both predator species the same limited ration, we took advantage of known bioenergetic information that enabled us to estimate species-specific limited rations equaling 50% of maximum consumption rates (C_{max}). Specifically, the amount of prey (weight (g)) fed to single-species treatments was equal to 50% of the estimated maximum consumption based on size and species (bluefish or striped bass) of the six individual fish in each mesocosm. Each mixed-species mesocosm received amounts of prey equal to 50% of the estimated maximum consumption of three bluefish and three striped bass. The maximum consumption rates we used were based on relationships between C_{max}, fish size, and water temperature determined experimentally for bluefish and striped bass by Hartman and Brandt (1995*b*). Ration levels were adjusted every 10 days based on updated predator size data. Each treatment mesocosm was fed weighed amounts of live fish prey daily (Atlantic silversides and mummichog (*Fundulus heteroclitus*)). Dead fish prey were removed and weighed daily. Mortalities of bluefish (n = 1) and striped bass (n = 1) occurred between days 10 and 20 and were replaced with fin-clipped fish of similar size. The new additions kept any density-dependent influences on feeding and growth rate constant throughout the experiment. Growth of the original fish was monitored separately from the new additions.

Fig. 1. Diet data pooled across prior studies in New York Bight estuaries for (*a*) age-1 striped bass and (*b*) age-0 bluefish), illustrating utilization of fish (solid bars) and invertebrate (open bars) prey resources during summer and early fall months. Ranges in total length by month across years are as follows: age-1 striped bass: May (77–261 mm), June (78–207 mm), July (96–241 mm), August (96–241 mm), September (164–296 mm), and October (164–296 mm); age-0 bluefish: June (51–99 mm), July (59–176 mm), August (69–256 mm), and September–October (118–260 mm). Size range data by month were not available in Gardinier and Hoff (1982).



The mixed- versus single-species treatments were compared using repeated-measures ANOVA with weight as the dependent variable and day as the independent variable. The analysis was performed separately for bluefish and striped bass. The specific growth rate during the 50-day experiment was calculated as (ln Wt_f – ln Wt_i)/d × 100, where Wt_f is final wet weight (g), Wt_i is initial wet weight (g), and d is number of days. Means and standard errors for specific growth were calculated for each treatment. Prey availability was indexed for each treatment as the average biomass of fish prey available to individual predators and was calculated as the biomass of fish prey fed to each mesocosm over the 50-day experiment divided by the number of predators. Prey fish availability in single- versus mixed-species treatments and the observed growth rates in the single-species treatments were used to formulate expected growth patterns for the mixed-species treatment.

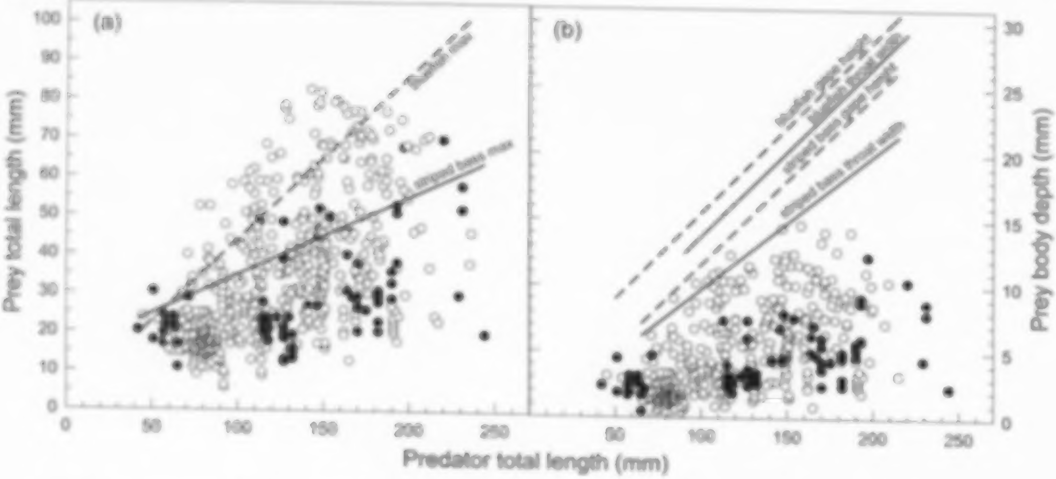
Results

Diet, prey size, and predator morphology

When examined synoptically across multiple summer–fall periods, age-0 bluefish diets were consistently dominated by fish, while age-1 striped bass included considerably more invertebrate prey in their diets (Fig. 1). Striped bass showed a trend of increased fish prey utilization during late summer and early fall, which coincided with increasing predator body size. In contrast, bluefish consistently foraged mainly on fish prey from the outset of summer through fall. The collective diet data confirm that age-0 bluefish demonstrate an earlier onset of piscivory and consume considerably more fish prey compared with similarly sized age-1 striped bass in New York Bight estuaries.

When feeding on bay anchovy and Atlantic silversides,

Fig. 2. (*a*) Prey total length versus predator total length (PredTL) for bluefish (○) and striped bass (●) feeding on bay anchovy and Atlantic silversides (both prey species pooled). The 90th quantiles for bluefish (Max prey = 0.42PredTL + 1.60) and striped bass (Max prey = 0.20PredTL + 14.71) represent the relationship between maximum prey size consumed and predator size. (*b*) Prey body depth (bay anchovy and Atlantic silversides pooled) and gape allometries as a function of PredTL. Allometric equations: bluefish gape height = 0.13PredTL + 2.43, $r^2 = 0.89$; bluefish throat width = 0.13PredTL + 0.84, $r^2 = 0.92$; striped bass gape height = 0.13PredTL – 1.27, $r^2 = 0.99$; striped bass throat width = 0.10PredTL – 0.46, $r^2 = 0.99$. Bluefish gape height was originally reported in Juanes and Conover (1995); bluefish throat width was originally reported in Scharf et al. (2002).



maximum prey sizes consumed in the field by bluefish increased at a faster rate (steeper slope) with increasing predator size compared with maximum prey sizes eaten by striped bass (Fig. 2*a*; comparison of quantile regression slopes: $F = 11.09$; $P < 0.001$). Thus, at a given predator body size, bluefish were able to consume substantially larger prey than striped bass across the range of body sizes examined. Allometric relationships indicated that predator gape size did not represent a morphological limitation for maximum prey size, since body depths of bay anchovy and Atlantic silverside prey were smaller than maximum ingestible prey sizes predicted from predator gape allometries, especially for larger predators (Fig. 2*b*). Although bluefish gape sizes at a given body length were larger compared with striped bass ($\text{ANCOVA}_{\text{gape height}}$: $F = 131.39$, $P < 0.001$; $\text{homogeneity of slopes}_{\text{throat width}}$: $F = 10.88$, $P < 0.005$), striped bass were clearly morphologically capable of ingesting larger prey sizes than those observed in their diet.

Laboratory experiments

Size-dependent foraging ability and behavior

Bluefish capture success was significantly higher compared with striped bass for both prey fish species ($\text{ANCOVA}_{\text{bay anchovy}}$: $F = 87.56$, $P < 0.001$; $\text{ANCOVA}_{\text{Atlantic silverside}}$: $F = 65.58$, $P < 0.001$). Differences in percent capture success demonstrated that bluefish were often two–three times more likely to capture a given prey fish, with increasing disparities between predators at larger relative prey sizes (Fig. 3*a*). Striped bass handling time increased at a significantly faster rate for larger relative prey sizes compared with bluefish for both prey fish species ($\text{homogeneity of slopes}_{\text{bay anchovy}}$: $F = 26.81$, $P < 0.001$; $\text{homogeneity of slopes}_{\text{Atlantic silverside}}$: $F = 7.81$, $P = 0.007$). Prey fish handling times were generally similar for bluefish and striped bass feeding on either prey fish species at small to intermediate relative body sizes; however, striped bass experienced much longer handling times for larger relative prey sizes (Fig. 3*b*). For both prey fish species, peak bluefish profitability was close to four times higher than that for striped bass and was located at substantially larger relative prey sizes (Fig. 3*c*).

When feeding on sand shrimp prey, striped bass capture success was high (40%–60%) at small relative prey sizes (0.10–0.20), but declined rapidly to 25%–30% above relative prey sizes of 0.20 and was close to zero above relative prey sizes of 0.30. The time required for striped bass to handle sand shrimp prey also increased considerably as relative prey sizes increased above 0.20 and was greater than the time required to handle fish prey at all sizes. Striped bass profitability when feeding on shrimp peaked at lower relative prey sizes and was lower compared with feeding on fish prey at all relative prey sizes above 0.15. Similarly, data presented in Juanes et al. (2001) indicated that bluefish realized higher capture success, lower handing times, and higher profitability when feeding on fish prey compared with similarly sized sand shrimp prey.

Both predator species schooled tightly during feeding experiments, with bluefish generally occupying the middle to upper water column and striped bass staying mainly in the lower portions of the feeding arena. Both predator species frequently attacked solitary prey individuals that had become separated from prey schools and approached prey at rapid speeds. Maximum attack velocities for bluefish were significantly higher compared with striped bass within the range of body sizes tested (ANCOVA: $F = 49.29$, $P < 0.001$). Maximum bluefish attack velocities ranged from 150 to 225 cm·s^{-1}, while maximum attack velocities for striped bass were between 125 and 160 cm·s^{-1} (Fig. 4).

Fig. 3. (*a*) Capture success, (*b*) mean predator handling time, and (*c*) profitability (defined in Materials and methods) of bluefish (solid lines) and striped bass (dashed lines) when feeding on common estuarine fish prey (bay anchovy and Atlantic silversides). The single dotted line in each panel represents the relationship for striped bass foraging on sand shrimp. Comparable data for bluefish feeding on sand shrimp are presented in Juanes et al. (2001), but could not be added to this figure, since experimental animals were categorized only as small or large, and thus exact sizes of individuals were not recorded for each trial. Least squares regression lines presented are based on analysis of all foraging trials completed. Note the difference in the scale of the *x* axis for panel (*c*).

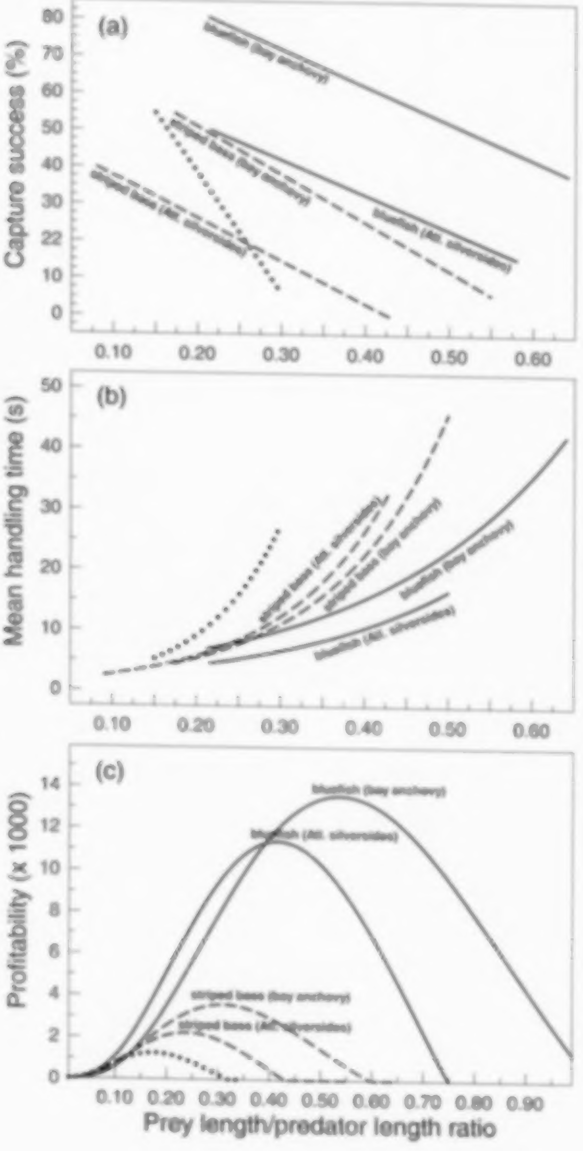
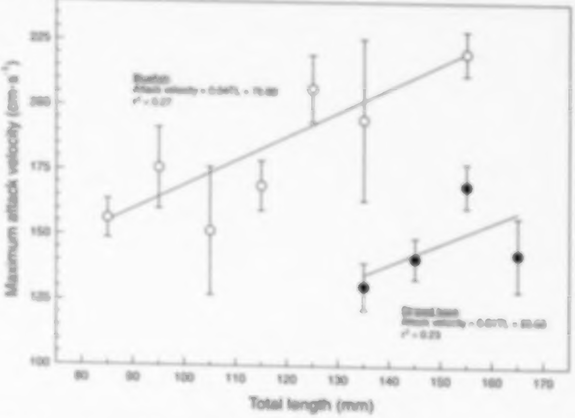


Fig. 4. Maximum attack velocity for bluefish (○) and striped bass (●) predators. Means and standard errors for each 10 mm total length (TL) range are indicated.



Predator growth on shrimp versus fish diet

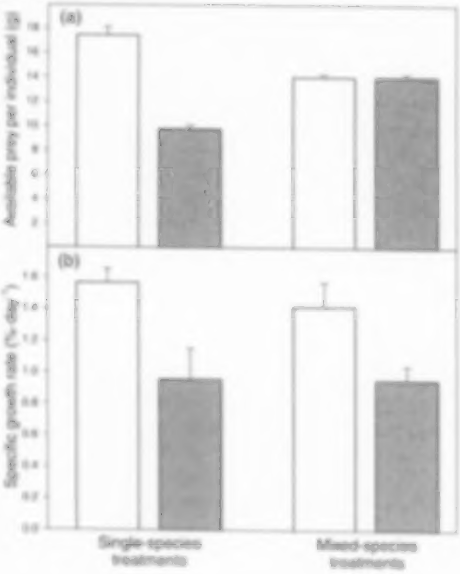
The initial mean weights (± standard error, SE) of striped bass in the shrimp- and fish-fed treatments were 40.0 g (±0.7 g) and 40.9 g (±2.3 g), respectively, and did not differ among treatments (t = –0.399, df = 4, P = 0.710). After 20 days, repeated measures ANOVA revealed a marginally significant (interaction term, P = 0.057) difference in growth of striped bass fed a fish versus a shrimp diet. Striped bass in fish-fed tanks were an average of 5.2 g heavier at the end of the experiment than striped bass that were fed a shrimp diet. Fish-fed striped bass had an average instantaneous growth rate of 0.017 (1.7%·day^{-1}) compared with an instantaneous growth rate of 0.013 (1.3%·day^{-1}) for shrimp-fed striped bass, a difference of 30%. Previously published experimental results for bluefish demonstrated that when fed a fish diet, bluefish were more than 16 g heavier, on average, at the end of 60 days than bluefish fed a shrimp diet (see Buckel et al. 2004 for experimental details). Bluefish instantaneous growth rates were 0.041 (4.1%·day^{-1}) on a fish diet and 0.032 (3.2%·day^{-1}) on a shrimp diet, a difference of 28% (Buckel et al. 2004). Both bluefish and striped bass realized higher growth rates when consuming piscine prey compared with shrimp prey.

Foraging ability in a mixed-predator assemblage

The initial mean weights (±SE) of bluefish were 26.2 g (±0.8 g) and 27.8 g (±0.7 g) in the single- and mixed-species treatments, respectively. Initial mean weights of striped bass were 25.3 g (±1.3 g) in the single-species treatments and 28.3 g (±1.6 g) in the mixed-species treatments. Values of initial mean weight did not differ within species for the mixed- versus single-species treatments (bluefish: t = –1.53, df = 4, P = 0.201; striped bass: t = –1.48, df = 4, P = 0.212) and also did not differ between species within the mixed-species treatment (t = –0.27, df = 4, P = 0.802). Temperature and salinity were stable throughout the 50-day experiment (mean temperature = 19.9 °C, range = 17.7–21.5 °C; mean salinity = 23.6 ppt, range = 21.8–25.5 ppt).

To interpret the growth data, expected growth rates in the mixed-species treatments were based on the absolute amount

Fig. 5. Mean (+1 standard error, SE) (*a*) prey fish biomass available per individual predator and (*b*) specific growth rate for bluefish (open bars) and striped bass (solid bars) in single- and mixed-species treatments during foraging ability experiment. Under the assumption of no difference in foraging ability between predator species and equal amounts of prey biomass available per individual in the mixed-species treatment, we hypothesized growth in the mixed-species treatments would be lower for bluefish and higher for striped bass relative to observed growth rates for each species in the single-species treatments.



of prey available (Fig. 5*a*) and observed growth in the single-species treatments (left side of Fig. 5*b*). We limited rations to 50% of per capita ($g \cdot g^{-1} \cdot day^{-1}$) maximum consumption, which differs between bluefish (~10% body mass per day) and striped bass (~6.5% body mass per day) based on bioenergetic experiments conducted for these life stages (Hartman and Brandt 1995*b*). Therefore, the three treatments received different absolute amounts of prey over the course of the experiment, necessitating that expected growth rates for the mixed-species treatment were based on these differences coupled with observed growth in the single-species treatments (Fig. 5). On average, bluefish in mixed-species treatments had access to less prey per individual compared with bluefish in single-species treatments, and striped bass in mixed-species treatments had access to more prey per individual compared with striped bass in the single-species treatments. Thus, the following growth rate predictions were made for the mixed-species treatment assuming equal foraging abilities of bluefish and striped bass: bluefish in the mixed-species treatment would grow slower than bluefish in the single-species treatment, while striped bass in the mixed-species treatment would grow faster than striped bass held in the single-species treatment.

Both bluefish and striped bass grew significantly over time (Table 1, Fig. 5*b*). However, we detected no significant differences in growth of bluefish or striped bass between single- and mixed-species treatments (Table 1, Fig. 5*b*). Observed growth rates in the mixed-species treatment did not match expectations that assumed equivalent foraging abilities; bluefish grew faster than expected, while striped bass grew more slowly than expected. To produce the observed growth pattern, bluefish would have required nearly two-thirds of the available prey in the mixed-species treatment. Therefore, when held in mixed-predator assemblages, bluefish displayed greater foraging ability and were able to exploit prey fish resources at the expense of striped bass in a limited prey environment.

Table 1. Repeated measures analysis of variance with treatment (single- versus mixed-species) and time as independent variables and weight as the dependent variable for bluefish and striped bass.

Effect	df	SS	*F*	*P*
Bluefish weight				
Treatment	1	4.52	0.13	0.735
Time	5	3808.71	438.93	<0.0001
Treatment × time	5	12.90	1.49	0.239
Striped bass weight				
Treatment	1	199.34	4.09	0.113
Time	5	1073.26	148.36	<0.0001
Treatment × time	5	6.07	0.84	0.537

Note: Nonsignificant *P* values for the treatment × time interaction terms indicates no statistical difference in growth trajectories between single- and mixed-species treatments for each predator species.

Discussion

Patterns of resource utilization

Our synoptic examination of diet data from New York Bight estuaries indicates that age-0 bluefish become piscivorous earlier and consume more fish prey compared with age-1 striped bass of similar size. The feeding habits of bluefish and striped bass in New York Bight estuaries are similar to patterns observed for these species in other US Atlantic coast estuaries located at northern latitudes. Age-0 bluefish diets consist mainly of fish prey upon estuarine arrival in early summer (Juanes and Conover 1995; Buckel and Conover 1997; Buckel et al. 1999), while age-1 striped bass diets are dominated by invertebrates in early summer with a trend towards increased piscivory in late summer and early fall (Schaefer 1970; Gardinier and Hoff 1982; Buckel and McKown 2002).

Variation in the onset of piscivory has been documented in freshwater systems both among piscivorous species and within a single species across years and systems (Keast 1985; Mittelbach and Persson 1998; Graeb et al. 2005). Among freshwater piscivores, some appear structurally adapted for an early shift to piscivory, while others do not begin eating fish prey until much later in life (termed "specialist" and "secondary" piscivores, respectively, by Keast 1985). The early onset of piscivory in age-0 estuarine bluefish would characterize them as a specialist piscivore; however, striped bass are difficult to categorize. The secondary piscivores in freshwater systems studied by Keast (1985) generally did not include considerable amounts of fish prey in the diet until ages 3 or 4. The striped bass in New York Bight estuaries, in contrast, typically include moderate amounts (up to 30% FO) of fish prey in their diets by the fall of age 1 and have been observed in some instances to eat small amounts (<10% FO) of fish prey during their first year (age-0 fish) (Buckel and McKown 2002; Jordan et al. 2003). Although the trend for striped bass at northern latitudes indicates a gradual increase in piscivorous feeding during their second and third summers (age-1 and age-2 fish), considerable variation in the timing and extent of piscivory in striped bass has been observed along the US Atlantic coast. In the Chesapeake Bay, located south of the New York Bight, fish prey constituted >60% of the biomass of prey eaten by age-1 striped bass by midsummer (Hartman and Brandt 1995*a*). Similarly, Markle and Grant (1970) found that fish occurred in >20% of the stomachs containing food and represented >50% by volume of the diet of juvenile striped bass between 30 and 150 mm TL from three major tributaries in the southern region of Chesapeake Bay. Moreover, striped bass appear to make an even earlier shift to piscivory at more southern latitudes, as age-1 striped bass in the Albemarle Sound estuary, North Carolina, were found to be completely piscivorous (>95% FO and % weight) by June of their second summer during two independent studies separated by more than 30 years (Manooch 1973; Rudershausen et al. 2005). Larger body sizes at age-1 for striped bass at southern latitudes (Rudershausen et al. 2005) provide a greater size advantage over piscine prey and may be mostly responsible for an earlier shift to a piscivorous diet in these systems.

In addition to including greater proportions of fish in their diet, we found that for a given predator body size, bluefish were able to consume larger sizes of identical prey species compared with striped bass. Since some of our bluefish prey size data was compiled from different years and estuaries than striped bass prey size data, spatial and temporal differences in prey size availability could partially explain these differences. However, based on the similarity in relative prey sizes eaten by striped bass in estuaries outside of New York Bight, this explanation does not appear likely. Manooch (1973) and Rudershausen et al. (2005) each found that striped bass consumed small relative prey sizes (prey/predator size ratios (PPR) = 0.13–0.27 (Manooch 1973); PPR = 0.19–0.27 (Rudershausen et al. 2005); mean PPR = 0.21 for both studies) in Albemarle Sound, North Carolina. These relative prey sizes were nearly identical to those observed for age-1 striped bass in New York Bight estuaries, suggesting that juvenile striped bass along the US Atlantic coast consume mostly small prey relative to their body size that are smaller than the relative prey sizes eaten by juvenile bluefish.

Morphological and behavioral explanations

Factors that can influence the timing and extent of piscivory may often do so indirectly through their effect on predator–prey body size relationships and include timing of spawning of predator and prey species, water temperature, growth rates during the invertebrate feeding stage, and predator and prey fish densities. To ensure the size advantage necessary to achieve piscivory, Keast (1985) noted that specialist freshwater piscivores spawned sufficiently earlier than their piscine prey. Indeed, bluefish utilize warm waters off the southeastern US continental shelf to spawn ahead of, and gain a size advantage over, their primary prey species, which are restricted to later spawning periods in more northern estuaries (Juanes and Conover 1995). This spawning strategy enables age-0 bluefish to feed on fish prey during their first summer. However, age-1 striped bass also achieve a considerable size advantage over potential prey fishes, since they are spawned a full year ahead of the forage fish available to them during their second summer of life. In fact, age-1 striped bass are larger than age-0 bluefish when they each enter estuaries in early summer, an indication that the difference in spawning timing should not be a primary factor explaining disparities in consumption of fish prey between these predators.

Gape allometry also did not appear to limit maximum prey sizes that predators were able to consume. Both predators possessed sufficiently large gapes to consume larger prey than were included in their diets, indicating that the sizes of forages fishes eaten were not morphologically constrained. Several studies have noted the importance of predator gape allometry in regulating prey sizes eaten (Persson et al. 1996; Scharf et al. 2000). However, evidence is equally available indicating that the sizes of prey eaten by many predators do not approach gape limitations, with limits instead being potentially set by prey behavior and availability (Gaughan and Potter 1997; Scharf et al. 2000; Galarowicz et al. 2006). In their review, Mittelbach and Persson (1998) found freshwater piscivores with larger mouth gapes switch to piscivory at smaller sizes and were larger at age-1. For bluefish and striped bass, gape sizes did not appear to be responsible for observed differences in the timing and extent of piscivory.

Foraging abilities of bluefish and striped bass exhibited size-dependent patterns typical of piscivorous fish predators, with linear declines in capture success, exponential increases in handling time, and dome-shaped patterns of profitability as relative prey size increased. However, there were striking differences in foraging ability between piscivore species. Bluefish capture success was generally two–three times higher than striped bass capture success on equal sized prey. Differences were greatest at large relative prey sizes, implying that the availability of relatively small forage fishes is likely of paramount importance to the ontogeny of piscivory in striped bass. The rapid increases in prey handling time demonstrate the potential for increased foraging costs to striped bass when prey sizes are large. Using large amounts of time to handle prey can be energetically costly and may also increase the risk of interactions with potential predators and competitors (e.g., Nilsson et al. 2000). When feeding on sand shrimp prey, both predator species realized lower profitabilities compared with feeding on similar-sized fish prey, an indication that neither species obtains an advantage in foraging efficiency when feeding on a common benthic invertebrate prey. Reduced foraging efficiency and growth rate in bluefish and striped bass when feeding on shrimp prey combine to suggest that there should be strong selection for an accelerated shift to piscivory in both predators.

Profitability functions serve as an index of predator feeding efficiency and probably constitute the most complete measure of piscivore foraging ability when prey are readily available. The measure does not account for differences in predator encounter rates with potential prey that may be related to differences in predator search strategy, activity level, or visual acuity, but rather combines capture success and handling time functions with the mass and energetic content of prey ingested to generate a relative estimate of size-dependent feeding efficiency. We assumed that search costs were negligible and encounter probabilities were essentially 100% in our experiments, regardless of predator and prey species or size, because of the moderate tank volumes, abundant light, and high water transparency (Mazur and Beauchamp 2003; Turesson and Brönmark 2007). For each prey fish species, peaks in bluefish profitability were nearly four times higher than those of striped bass, indicating that bluefish are more efficient at capturing and manipulating fish prey for ingestion. More importantly, bluefish profitability peaked at larger relative prey sizes compared with striped bass, illustrating the ability of bluefish to forage effectively on even relatively large fish prey and further establishing the limitations for striped bass when small forage fishes are not available.

Behavioral observations revealed that bluefish and striped bass employed similar attack strategies, but that bluefish attained substantially higher burst velocities when attacking prey compared with striped bass. Strike tactics of both predators involved approaching prey at low swimming velocities (30–40 cm·s^{-1}) followed by rapid acceleration from an "S-start" position to strike at prey with high velocity (125–200 cm·s^{-1}). Webb (1984) observed variation in strike speeds among predators employing different attack strategies and concluded that predators likely optimized attack speeds to minimize closure times. Bluefish and striped bass attack behaviors did not exhibit obvious differences; thus, higher strike velocities by bluefish may have contributed to higher capture efficiencies. Moreover, striped bass swimming velocity often peaked slightly ahead of the strike, which may have negatively affected prey capture success. Bluefish tooth morphology could also contribute to their greater foraging ability. Bluefish possess large, sharp, deeply socketed teeth, a suite of dentition characters shared by other vertebrate groups that display extreme carnivory on large, active prey (Bemis et al. 2005). Tooth structure and attachment in bluefish allow larger prey to be severed into smaller pieces, whereas striped bass have small cardiform teeth and must swallow prey whole. The ability of bluefish to sever prey also reduces handling time on larger prey (Scharf et al. 1997), thus increasing overall foraging efficiency.

Consequences for predator growth

Growth rates of fishes can increase considerably after switching from an invertebrate to a fish diet (Buijse and Houthuijzen 1992; Buckel et al. 1998; Galarowicz and Wahl 2005). We found about a 30% increase in specific growth rate for age-1 striped bass fed a fish diet versus a shrimp diet, and we believe that the marginal statistical significance was mostly a result of the short duration of the experiment. A previous study (Buckel et al. 2004) demonstrated a similar pattern for juvenile bluefish and observed a 28% increase in specific growth rate for bluefish that were fed fish compared with those fed shrimp. Thus, both bluefish and striped bass suffer lower growth rates during the juvenile period when their diet is dominated by a common invertebrate prey. Friedland et al. (1988) observed lower condition in field-captured age-0 bluefish during summers when benthic invertebrates (mostly small shrimp) were more prevalent in the diet and concluded that benthic invertebrates likely represented an energy-poor prey relative to fish. Indeed, mean energy content has been determined to be considerably lower for sand shrimp (3.7 kJ·g wet weight^{-1}) than bay anchovy (5.9 kJ·g^{-1}) or Atlantic silversides (7.3 kJ·g^{-1}) (Steimle and Terranova 1985) and contributes to lower profitability.

In an earlier study, Buckel and McKown (2002) found evidence for resource (habitat and diet) partitioning between bluefish and striped bass in New York Bight estuaries and concluded that resource partitioning could have resulted from past (sensu Rosenzweig 1981) competition (interference or exploitative) or that it could be an indication that these two species do not compete. Their laboratory experiments with mixed-predator assemblages and unlimited prey confirmed that interference competition was unlikely between these two species; however, they did not examine resource utilization under limited prey conditions. In this study, using limited prey rations determined bioenergetically, we observed that bluefish consumed nearly two-thirds of the available forage and thus were able to exploit prey fish resources at the expense of striped bass in a limited fish prey environment. Our findings suggest that exploitative competition is a potential mechanism leading to differences in habitat and prey use in New York Bight estuaries; future field studies should further investigate this possibility.

Divergent patterns of resource utilization are often caused by differences in foraging abilities between sympatric predators. For example, juvenile roach (*Rutilus rutilus*) attack and capture zooplankton prey more efficiently than European perch (*Perca fluviatilis*), leading to greater resource limitation, reduced growth, and lower survival in perch when the two species co-occur in lake systems with depressed prey resources (Persson et al. 2000). Eklöv and Diehl (1994) concluded that growth differences between piscivorous adult perch and northern pike (*Esox lucius*) fed fish prey and held separately under identical conditions resulted from differences in predator morphology, attack efficiency, and behavior; however, the authors concluded that differences may be altered if pike and perch were held together in mixed-predator assemblages. The differences in foraging ability that we found likely contribute to disparities in growth between juvenile bluefish and striped bass observed in natural systems. Long-term (1986–1998) field data in New York Bight estuaries demonstrate consistently lower growth rates for age-1 striped bass ($\bar{x}$ = 0.95 mm·day^{-1}) compared with age-0 bluefish ($\bar{x}$ = 1.43 mm·day^{-1}) during summer months (K.A. McKown, unpublished data). Higher estuarine growth rates observed for age-0 bluefish compared with age-1 striped bass are likely due primarily to an earlier shift to piscivory by bluefish, which is facilitated to a large degree by their exceptional foraging abilities when feeding on fish.

The consistently lower growth rates and large non-fish component of age-1 striped bass diets suggest that striped bass have limited access to fish prey during their second summer in northern US Atlantic coast estuaries. We stress that access to fish prey resources in these systems is not necessarily limited by low absolute densities of forage fishes, but rather is restricted by having access to only a small fraction of the available prey fish size distribution. Juvenile striped bass should feed most efficiently on prey fishes that are 20%–30% of their body size, mainly because of difficulty in capturing forage fishes at larger relative sizes. Age-1 striped bass begin their second summer at smaller body sizes in northern US Atlantic coast estuaries compared with systems further south (Rudershausen et al. 2005), meaning that the primary forage fish species (e.g., atherinids, clupeids, and engraulids) may quickly grow beyond the most vulnerable sizes. Research in freshwater systems has highlighted the importance of predator–prey body size relationships to the growth and survival of piscivores (Donovan et al. 1997; Cyterski and Ney 2005). Our findings suggest that latitudinal variation in diet and growth of juvenile striped bass are also influenced by predator–prey body size relationships, which ultimately determine prey fish availability.

Although the effect on diet of variable prey densities was not examined in this study, the relative densities of benthic invertebrate and fish prey may also contribute to delayed piscivory in striped bass. In early summer, sand shrimp densities can be considerably higher than densities of forage fishes in northern US Atlantic coast estuaries, leading to higher encounter rates with shrimp prey and inclusion in predator diets (Juanes et al. 2001). However, even when fish prey were at lower relative densities, juvenile bluefish showed positive selection for fish prey and negative selection for shrimp that was related mainly to higher bluefish capture success on fish prey (Juanes et al. 2001). We discounted our profitability index by multiplying by the percentage of successful capture attempts, but the actual energetic consequences of failed strikes may represent a much greater cost. At small relative body sizes (10%–20%) in early summer, shrimp prey may represent a relatively profitable prey for juvenile striped bass because of high encounter rates and their relative ease of capture compared with larger fish prey. Estimates of the energetic costs of failed capture attempts (e.g., Selch and Chipps 2007) would enable a greater mechanistic understanding of the patterns of prey selection by bluefish and striped bass.

We conclude that contrasting patterns of resource utilization in northern US Atlantic coast estuaries between these two predators may be driven largely by differences in foraging ability. Juvenile striped bass have limited capture abilities when prey fishes are relatively large; thus, piscivory is delayed until the relative size of prey fishes is reduced through predator growth, increased availability of relatively small prey fishes, or a combination thereof. The early shift to piscivory observed for juvenile striped bass at more southern latitudes indicates that this species will take advantage of the accelerated growth provided by a piscivorous diet if prey fish sizes are small relative to predator size. Our findings highlight the importance of the availability of appropriately sized forage fishes to the ontogeny of piscivory and provide evidence that predator–prey size relationships and disparate foraging abilities can generate variation in patterns of resource utilization and predator growth. The important growth consequences of patterns of resource utilization by piscivorous fishes call for more rigorous assessments of forage fish size distributions and quantitative evaluation of foraging behavior to identify the processes that contribute to inter- and intra-specific variation.

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Evidence of density- and size-dependent mortality in hatchery-reared juvenile white sturgeon (*Acipenser transmontanus*) in the Kootenai River

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Abstract: We evaluated effects of stocking level and size-at-release on survival rates of hatchery-reared juvenile white sturgeon (*Acipenser transmontanus*) in the Kootenai River using Cormack–Jolly–Seber and related models implemented in Program MARK. A total of 119 768 marked and unmarked hatchery juveniles were released from 1992 to 2006, of which 2938 passive integrated transponder (PIT)-tagged fish were subsequently recaptured. Annual survival rates of marked groups ranged from 0.01 to 0.84 (mean = 0.45) during the first year at large, from 0.48 to 1.0 (mean = 0.84) in the second year, and averaged 1.0 during all subsequent years. First year survival rates declined substantially in recent years, particularly for small fish (<25 cm fork length). Approximately 59% of the variation in first year survival was explained by a negative relationship with estimated juvenile abundance (linear regression, $P < 0.01$). Length-at-release of individuals explained a substantial proportion of the within-year variation in survival during the first year at large. Our results provide strong evidence of density- and size-dependent mortality in hatchery-reared juvenile white sturgeon in the Kootenai River. Management actions that prioritize the release of fewer, larger-sized fish will likely improve first year survival rates and subsequent recruitment to the spawning-age population.

Résumé : Nous évaluons les effets de l'intensité de l'empoissonnement et de la taille à la libération sur les taux de survie de jeunes esturgeons blancs (*Acipenser transmontanus*) de pisciculture dans la Kootenai à l'aide du modèle Cormack–Jolly–Seber et de modèles apparentés utilisés dans le logiciel MARK. Un total de 119 768 jeunes de pisciculture marqués et non marqués ont été libérés de 1992 à 2006, dont 2 938 poissons porteurs d'étiquette PIT (à transpondeur intégré passif) ont été recapturés plus tard. Les taux de survie des groupes marqués variaient de 0,01–0,84 (moyenne = 0,45) durant la première année en liberté, de 0,48–1,0 (moyenne = 0,84) la seconde année et en moyenne de 1,0 durant toutes les années subséquentes. Les taux de survie durant la première année ont diminué considérablement au cours des dernières années, particulièrement chez les petits poissons (longueur à la fourche < 25 cm). Environ 59 % de la variation de la survie pendant la première année s'explique par une relation négative avec l'abondance estimée des jeunes (régression linéaire, $P <$ 0,01). La longueur des individus à la libération explique une partie importante de la variation intra-annuelle dans la survie durant la première année de liberté. Nos résultats apportent de fortes indications de l'existence d'une mortalité reliée à la densité et à la taille chez les jeunes esturgeons blancs de pisciculture dans la Kootenai. Des activités de gestion qui favoriseraient la libération d'un nombre moins important de poissons de plus grande taille augmenteraient vraisemblablement les taux de survie pendant la première année et le recrutement subséquent dans la population en âge de frayer.

[Traduit par la Rédaction]

Introduction

A long individual life span has forestalled extinction of the Kootenai River white sturgeon (*Acipenser transmontanus*), listed in 1994 as endangered under the US Endangered Species Act (Duke et al. 1999). Natural recruitment began declining around 1960, and the last substantial year class was produced in the early 1970s (Andrusak 1980; Partridge 1983). Kootenai River white sturgeon continue to migrate upriver and spawn each year, but mortality of eggs and larvae is nearly absolute (Duke et al. 1999; Paragamian et al. 2001; Paragamian and Wakkinen 2002). The wild population now consists of a dwindling cohort of large, old fish (Paragamian et al. 2005).

Recruitment failure coincided with an accumulation of large-scale alterations of the Kootenai River system over the last 100 years, including extensive floodplain and watershed development, mainstem dam construction, reduced productivity, and shifts in the biological community across all trophic levels. Peak spring flows in the Kootenai River were greatly reduced following construction of Libby Dam

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in 1972. Unregulated spring flows ranged from approximately 1700 to 2800 $m^3 \cdot s^{-1}$ (Apperson and Anders 1991), while post-dam flows have ranged from only 250 to 450 $m^3 \cdot s^{-1}$ (Paragamian and Kruse 2001). Beginning in the late 1800s, extensive floodplain development, including construction of levees, drainage ditches, and pumping stations, reclaimed substantial portions of historical floodplain habitat (Northcote 1973; Redwing Naturalists 1996). By 1990, approximately 90% of the historical wetlands and riparian habitats had been isolated from the river by levees (Soults and Anders 2003). In addition, the reservoir formed by Libby Dam currently acts as a nutrient sink, retaining considerable quantities of nutrients and organic material (Daley et al. 1981; Snyder and Minshall 1996). Although a variety of research and recovery measures have been implemented over the years, all efforts to date have failed to restore natural recruitment of Kootenai River white sturgeon or to identify a feasible strategy for restoring natural recruitment (Paragamian and Beamesderfer 2004).

It now appears that the next generation of Kootenai River white sturgeon will depend on a conservation aquaculture program initiated in 1990, without which the species would have been doomed to extinction (Paragamian and Beamesderfer 2004). Mature, wild fish are captured each year during the spawning migration, artificially spawned, and their progeny are reared in one of two hatcheries for 1 or 2 years before release into the wild (Ireland et al. 2002*a*; Kootenai Tribe of Idaho 2007). Survival of initial experimental hatchery releases was high, averaging 60% for the first year following release and 90% in subsequent years (Ireland et al. 2002*b*). Growth and condition were often poor within the first few years but increased in survivors that adapted to natural conditions. Large numbers of hatchery fish have been released in the interim.

Long-term prospects for sustaining any sturgeon population with a conservation aquaculture program remain highly uncertain. Depleted natural populations are being widely supplemented with hatcheries (Schram et al. 1999; Runstrom et al. 2002; Smith et al. 2002). However, hatcheries are considered to be poor substitutes for natural production and a conservation option of last resort for Kootenai River white sturgeon (Duke et al. 1999; Paragamian and Beamesderfer 2004). Important concerns include population bottlenecks or hatchery selectivity that may reduce genetic diversity and fitness, density-dependent mortality factors, and other ecological interactions related to overseeding the current habitat capacity (National Research Council 1996; Jager 2005; Drauch and Rhodes 2007).

The optimum number of broodstock and hatchery releases necessary to recover Kootenai River white sturgeon populations has been the subject of considerable debate (Paragamian and Beamesderfer 2004). Managers are faced with a difficult dilemma with respect to maximizing genetic diversity and recruitment to the spawning population while also avoiding overseeding of available rearing habitat. This study examines some of the critical uncertainties concerning the effects of increased hatchery production on survival of juvenile white sturgeon in the Kootenai River. Our objectives were to assess recent survival rates of hatchery-reared juvenile white sturgeon released into the Kootenai River and to examine the effects of size-at-release, environmental conditions, and increased stocking densities on survival.

Materials and methods

Study site

The Kootenai River contains a unique headwater population of white sturgeon that has been isolated from other populations in the Columbia and Snake rivers for some 10 000 years by natural falls at river kilometre (rkm) 24 (Northcote 1973). The Kootenai River is one of the largest tributaries to the Columbia River, draining approximately 35 490 km^2. Originating in British Columbia (BC), the river flows south into Montana where flows are impounded at Libby Dam (rkm 352) forming Koocanusa Reservoir (Fig. 1). From Libby Dam, the river flows west and then northwest into Idaho and then turns north into BC and Kootenay Lake near rkm 120. Kootenay Lake is a large, natural, oligotrophic lake, but the water level is now regulated by an outlet dam. Kootenai River white sturgeon range from Kootenay Lake upriver to Kootenai Falls, Montana (rkm 310), but are now found predominantly in the meandering, low-gradient reach downstream from Bonner's Ferry and in Kootenay Lake (Kootenai Tribe of Idaho 2007).

Fish sampling

Hatchery-reared juveniles were first released into the Kootenai River in 1992, and annual releases have occurred since 1997. Fish were reared for approximately 1–2 years at the Kootenai Tribal Sturgeon Hatchery in Bonners Ferry, Idaho, and at the Kootenay Trout and Sturgeon Hatchery in Ft. Steele, British Columbia. Releases were broadly distributed throughout the river, with release locations ranging from within Kootenay Lake (rkm 75–120) to sites as far upriver as Kootenai Falls (rkm 307). Prior to release, most hatchery-reared fish were measured (fork length and total length), weighed, and implanted with an individually numbered passive integrated transponder (PIT) tag. Fish were also marked by removal of lateral scutes to identify the fish's brood year in case of tag loss. More detailed information about the aquaculture program including brood stock collection and mating practices, fish health, size and numbers of river releases, sampling effort, and growth and condition is provided in Kootenai Tribe of Idaho (2007).

For analysis purposes, we grouped hatchery releases by year of release. Release year was defined as 1 March through 28 February, ensuring that all winter releases were included in the same year. For example, fish released in December 2005 and January 2006 were both defined as 2005 releases. Limited numbers of recaptures, particularly in recent years, precluded estimation of separate survival rates for fish grouped by release season or rearing facility.

Recapture data were provided by an annual juvenile white sturgeon monitoring program initiated in 1993 by the Kootenai Tribe of Idaho (KTOI), Idaho Department of Fish and Game (IDFG), and the British Columbia Ministry of Environment (MOE). Sinking multifilament gill nets with variable mesh size (1.0–6.3 cm) were fished between June and September at sites distributed from Kootenay Lake to the head of Kootenai Valley just upriver of the Kootenai Tribal Hatchery (rkm 77–236). Sampling locations were selected to represent a variety of available habitat types distributed throughout the river. Sampling effort varied considerably across years, with the total number of gill net hours ranging from a low of 298.4 in 1995 to a high of 890.6 in 2001.

Fig. 1. Range of white sturgeon (*Acipenser transmontanus*) in the Kootenai River Basin (shaded). Inset shows location in western Canada and USA.

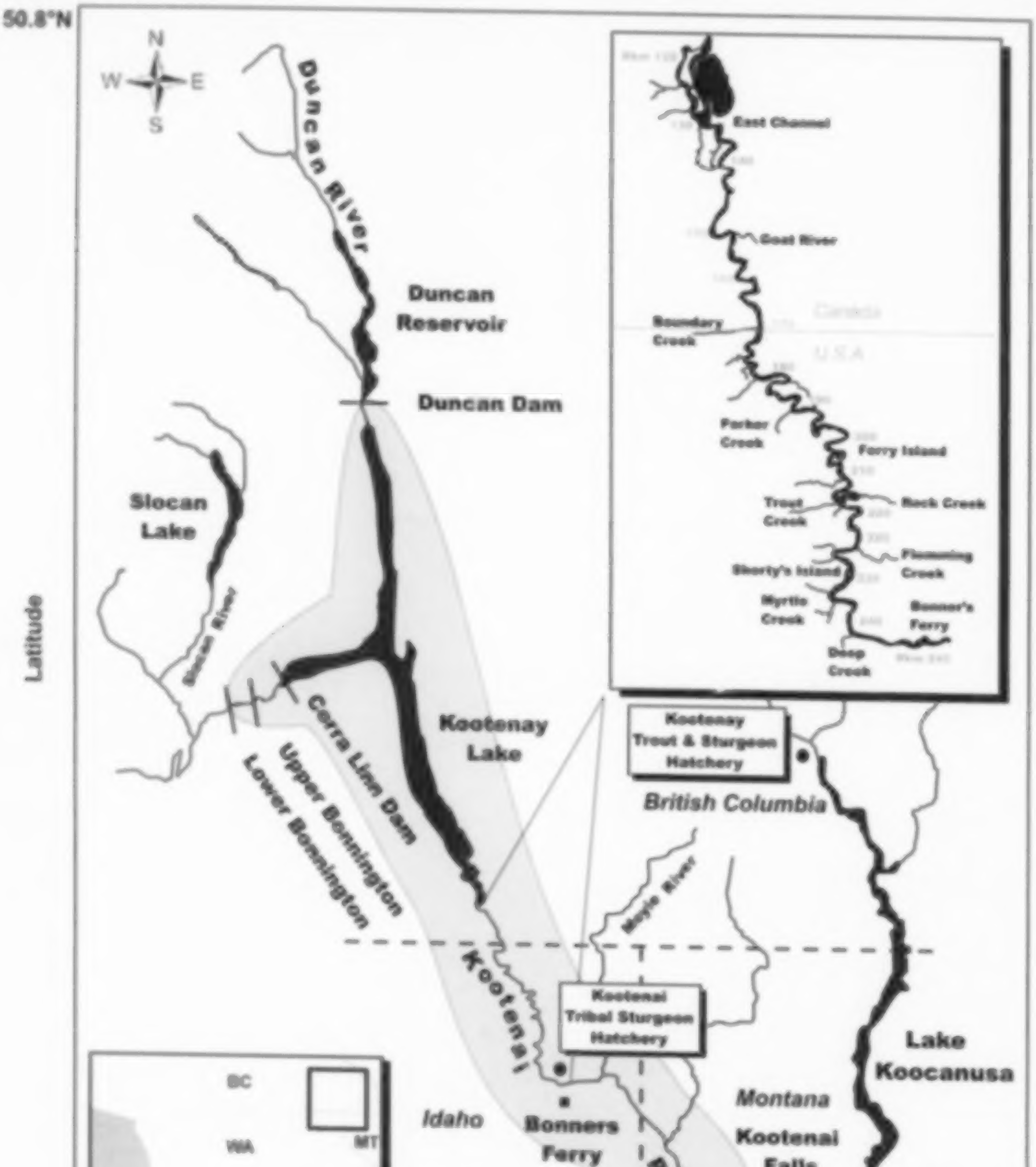

50.8°N
N
W
E
S
Duncan River
Duncan Reservoir
Duncan Dam
Slocan Lake
Slocan River
Corra Linn Dam
Upper Bonnington
Lower Bonnington
Kootenay Lake
Latitude
East Channel
Goat River
Canada
U.S.A
Boundary Creek
Parker Creek
Ferry Island
Trout Creek
Rock Creek
Flemming Creek
Shorty's Island
Myrtle Creek
Bonner's Ferry
Deep Creek
Kootenay Trout & Sturgeon Hatchery
British Columbia
Moyie River
Kootenai
Kootenai Tribal Sturgeon Hatchery
Lake Koocanusa
BC
WA
MT
ID
OR
Idaho
Bonners Ferry
Montana
Kootenai Falls
River
Libby Dam
Miles
5 10 15
0 10 20 30
Kilometres
48.3°N
117.7°W
Longitude
115.0°W

Table 1. Description of the nine noncovariate models (Models 1–9) and two length covariate models (Models 10–11) used to analyze capture–recapture data of juvenile white sturgeon (*Acipenser transmontanus*) released into the Kootenai River from 1992 to 2004.

Model	Model notation	Survival (φ)	Recapture (p)
1[a]	$\varphi(t), p(t)$	Year-specific (t)	Year-specific
2	$\varphi(a2\text{-}c/c), p(t)$	Two age classes (a; age-1 and age-2+); both constant (c) across years	Year-specific
3	$\varphi(a2\text{-}t/c), p(t)$	Age-1 year-specific Age-2+ constant	Year-specific
4	$\varphi(a2\text{-}t/t), p(t)$	Both ages year-specific	Year-specific
5	$\varphi(a3\text{-}t/c/c), p(t)$	Three age classes (age-1, age-2, and age-3+); age-1 year-specific, age-2 and age-3+ constant	Year-specific
6	$\varphi(a3\text{-}t/t/c), p(t)$	Age-1 and age-2 year specific, age-3+ constant	Year-specific
7	$\varphi(a3\text{-}t/t/t), p(t)$	All three age classes year-specific	Year-specific
8	$\varphi(a3\text{-}t/t/c), p(a2\text{-}t/t)$	Same as Model 6	Both ages year-specific
9[b]	$\varphi(a3\text{-}t/t/t), p(a2\text{-}t/t)$	Same as Model 7	Both ages year-specific
10	$\varphi(a3\text{-}l + t/t/c), p(t)$	Same as Model 6 but with a constant (additive) length covariate for age-1	Year-specific
11	$\varphi(a3\text{-}l \times t/t/c), p(t)$	Same as Model 6 but with a year-specific (multiplicative) length covariate	Year-specific

[a] Standard Cormack–Jolly–Seber model.

[b] Global model (i.e., most parameterized).

Survival rates

Survival rates of juvenile white sturgeon were estimated for PIT-tagged juveniles released and recaptured in the Kootenai River. Fish released into Kootenay Lake were excluded from the analysis because there was limited sampling in the lake and few recaptures of lake-released fish. In addition, we removed 17 fish from the data set that were missing critical release information such as brood year and length-at-release. We also excluded recaptures that occurred in the same year that a fish was released or previously recaptured.

Annual apparent survival rates (φ) and recapture probabilities (p) were estimated using Cormack–Jolly–Seber (CJS) and related models implemented in Program MARK (White and Burnham 1999). Apparent survival (hereby referred to as survival) is distinguished from true survival in that apparent survival combines the probability of survival and the probability of not permanently emigrating out of the study area (i.e., $\varphi = 1 - \text{mortality} - \text{emigration}$), whereas true survival deals only with mortality. Thus, in open populations, substantial levels of permanent emigration will result in CJS survival estimates that are biased low compared with true survival. Although it is possible that some river-released juvenile white sturgeon may have permanently emigrated out of the study area (such as into Kootenay Lake), such behavior is assumed to be limited and is a topic of current research in the basin. In its most general form, the CJS model is parameterized to produce estimates of survival and recapture probability for each sampling period. Variations of the general CJS model, such as models that allow for group effects (e.g., age classes) or individual covariates (e.g., fish length) can be implemented in Program MARK to test specific hypotheses.

We had two objectives in our modeling of survival rates of hatchery-reared juvenile white sturgeon. The first objective was to determine the basic age and time structure that best captured variation in survival rates and recapture probabilities. From a biological perspective, we expect that survival rates of released juveniles may be initially low as juveniles transition to the river environment, especially during their first year at large (Ireland et al. 2002*b*; Golder Associates, Ltd. 2006). In subsequent years, survival rates should increase rapidly and stabilize as fish grow larger, approaching high annual survival rates typically observed for sturgeon populations (McCabe and Tracy 1994; Gross et al. 2002). Following this biological framework, we examined an initial set of (noncovariate) capture–recapture models to estimate baseline survival rates as described below. Our second objective was to test for possible influences of body size-at-release on survival rates of juveniles. A priori, we might expect that larger size-at-release could increase the chance of survival among newly released fish via competitive advantages or reduced risk of predation (Sogard 1997; Mohler et al. 2000; Sutton and Ney 2001). Thus, once the baseline model structure was determined, we then examined two additional models with length covariates to explicitly test for effects of fish size-at-release on survival rates.

The initial set consisted of nine models that built on those previously examined by Ireland et al. (2002*b*). The first model was the standard CJS model, which assumed year-specific survival rates ($\varphi(t)$) and recapture rates ($p(t)$) (Model 1; Table 1). The next three models included two age classes for survival, where the first age class represented the first year at large (age-1 fish), and the second age class represented all subsequent ages (age-2+ fish) (Models 2–4; Table 1). The underlying hypothesis for these age-structured models was that survival during the first year following release differed substantially from survival in all subsequent years, as found by Ireland et al. (2002*b*). These age-structured models assumed constant survival for both age classes (Model 2); year-specific survival for age-1 fish and constant survival for age-2+ fish (Model 3); and year-specific survival for both age classes (Model 4). The remaining models included three age classes for survival: the first year at large (age-1 fish), the second year at large (age-2 fish), and a third age class representing all subsequent

Table 2. Number of hatchery-reared Kootenai River white sturgeon released from 1992 to 2006.

	British Columbia hatchery			Kootenai Tribal Hatchery			
Year	Tagged river releases	Tagged lake releases	Untagged river releases	Tagged river releases	Tagged lake releases	Untagged river releases	Total
1992	0	0	0	118	0	0	118
1993	0	0	0	0	0	0	0
1994	0	0	0	123	0	0	123
1995	0	0	0	0	0	0	0
1996	0	0	0	0	0	0	0
1997	0	0	0	1 959	0	0	1 959
1998	0	0	0	96	0	0	96
1999	0	0	0	334	0	0	334
2000	1 358	0	0	828	0	0	2 186
2001	3 237	0	0	2 777	0	0	6 014
2002	0	6 708	0	3 793	0	0	10 501
2003	0	7 579	0	8 382	0	0	15 961
2004	9 020	19	0	3 519	0	4 275	16 833
2005	1 238	0	4 240	0	0	32 560	38 038
2006	1 779	0	21 165	714	0	3 947	27 605
Total	16 632	14 306	25 405	22 643	0	40 782	119 768

ages (age-3+) (Models 5–9; Table 1). These models tested the hypothesis that survival rates differed during the first, second, and all subsequent years after release. The final two models assumed that recapture rates as well as survival rates were year-specific for both age classes (Models 8 and 9). These models allowed us to evaluate if recapture rates of age-1 fish differed from those of age 2+ fish, an effect that could result from size-selective gear effects. However, owing to limited statistical support for these models, investigation of additional models with three age classes for recapture probabilities was not warranted.

For models with year-specific survival rates for age-2 or older fish, we imposed parameter constraints to obtain viable estimates of age-1 survival for fish released in 1992. Because of limited sampling in 1993 and 1994, there were insufficient recaptures to estimate survival of 1992 releases in the first 2 years (no fish were released in 1993). Thus, where applicable, we set the survival rates for 1993 (age-2) and 1994 (age-3) to 1.0 (i.e., 100%), which allowed the age-1 survival rate for 1992 releases to be estimated using the observed recaptures in 1995. By fixing survival at 100% for 1993 and 1994, we assumed that all mortality between 1992 and 1994 occurred during the first year at large. Thus, the estimates for 1992 represent minimum estimates of age-1 survival. Also note that for CJS models, it is not possible to independently estimate survival and recapture probabilities for the last sampling period (Williams et al. 2002).

We selected the best fitting of the nine models using the quasi-Akaike information criterion (QAIC), which adjusts AIC by a variance inflation factor ($\hat{c}$) to account for potential overdispersion (Lebreton et al. 1992; Burnham and Anderson 2002). The QAIC model-selection criterion has been validated by simulation analyses (Anderson et al. 1994; Burnham et al. 1995) and is strongly recommended for capture–recapture studies (Lebreton et al. 1992; Burnham and Anderson 2002). Models were initially identified based on intuitive and biologically based structures. The strength of competing biological hypotheses was interpreted using the QAIC results, which indicate the relative support of these biological hypotheses (i.e., alternative model structures) given the data at hand. Our analysis and discussion of trends in Kootenai River white sturgeon survival focused on results from the QAIC-selected best-fitting model in the absence of irregular and biologically or statistically unreasonable results for that model.

To compute $\hat{c}$, we assessed the goodness-of-fit of the global model (i.e., the most parameterized model; Model 9) using a parametric bootstrap method (Cooch and White 2007) and calculated $\hat{c}$ as the ratio of the global model deviance over the mean deviance from 500 bootstrap simulated deviances. As an additional measure of performance, we computed the QAIC weight for each model, which measures as the weight of evidence in favor of a given model as the best fitting in the model set (Burnham and Anderson 2002).

Effect of size-at-release

To examine the influence of fish length-at-release on survival rates, we added length covariates to the best-fitting model from the initial model set (Model 6). Because estimates for Model 6 and other competing models indicated substantial variation in age-1 survival rates but relatively high and consistent survival for older age classes, we only examined length effects for the first age class. We used fork length-at-release (cm) as the individual covariate instead of total length or weight because this measurement was most consistently available in the data set. The logit link function was used in Program MARK to transform relationships between length and survival to a linear scale.

Using Model 6 as a baseline structure, we examined two models that included length-at-release as a covariate influencing age-1 survival. We began by adding a constant (additive) length effect for age-1 survival rates (Model 10; Table 1). This model assumed that age-1 survival varied across years but that the slope of the length effect was con-

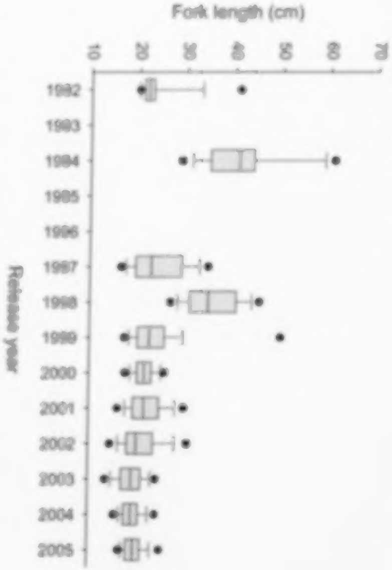
Table 3. Release and recapture information for passive integrated transponder (PIT)-tagged juvenile white sturgeon released by Kootenai hatchery programs (lake releases excluded), 1992–2005.

Releases				Recaptures															Individuals[a]	
Year	Lower rkm	Upper rkm	Total	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	Total	Total	%
1992	204	243	117	1	0	14	26	9	9	17	8	3	8	7	7	0	2	111	63	54%
1994	204	310	120			12	16	12	9	9	13	10	10	2	4	0	5	102	56	47%
1997	241	245	1 950						166	105	134	131	91	34	58	35	19	773	568	29%
1998	241	259	96							7	15	12	7	3	1	2	0	47	37	38%
1999	230	258	333								11	17	9	7	3	4	1	52	43	13%
2000	170	259	2 186									140	60	63	64	57	39	423	363	17%
2001	170	245	6 013										143	103	123	71	88	528	481	8%
2002	170	245	3 793											51	46	28	31	156	151	4%
2003	170	301	8 380												5	1	2	8	8	<1%
2004	144	307	12 539													64	81	145	142	1%
2005	151	275	1 238														5	5	5	<1%
Total			36 765	1	0	26	42	21	184	138	181	313	328	270	311	262	273	2 350	1 917	19%

Note: Release numbers vary slightly from those reported in Table 2 because some individuals with missing release information (i.e., length-at-release or brood year) were removed from the data set prior to estimating survival rates.

[a]Number and percentage of individuals from a release group that were recaptured at least once.

Fig. 2. Fork length-at-release (cm) for passive integrated transponder (PIT)-tagged juvenile white sturgeon by release year, 1992–2005. Box plots show medians (horizontal lines), 25th–75th percentiles (boxes), 10th–90th percentiles (whiskers), and 5th–95th percentiles (dots).



stant over time. The next model assumed a multiplicative relationship between length and time (Model 11); that is, the effect of length on age-1 survival varied across release years.

Again, we compared the models using QAIC to test the hypothesis that length-at-release influences survival of juvenile white sturgeon during their first year at large. Because no statistical procedure exists to evaluate goodness-of-fit of models that include individual covariates, we followed the recommended approach of using $\hat{c}$ as estimated for the global model excluding covariates (Model 9), as discussed above (Cooch and White 2007). We did not examine length effects for recapture probabilities of age-1 fish because there was little statistical support for age-class differences in recapture rates among noncovariate models.

To further examine the influence of fish length on survival, we compared size-at-release distributions for fish that were never recaptured with those for fish that were recaptured at least once. For each annual release group, we tested for differences in mean length-at-release between fish that were recaptured and not recaptured using a Wilcoxon rank sum test. We used this nonparametric test because size-at-release distributions were often non-normal.

Effect of environmental conditions and fish abundance

We used linear regression to evaluate relationships between age-1 survival rates and annual measures of environmental conditions and fish abundance by release year. Environmental variables included maximum annual river discharge, average annual river discharge, and average daily maximum temperature in August. The question was whether survival patterns were related to periodic floods or temperature extremes. Data on river discharge and temperature in the Kootenai River at Porthill, Idaho, were obtained from the US Geological Survey (USGS), National Water Information System (water.usgs.gov/). River discharge was summarized by release year (1 March – 28 February). Maximum daily river temperatures in each year were averaged for the month of August to correspond with the period when temperatures were warmest and most likely to affect juvenile

Table 4. Comparisons of the 11 capture–recapture models (Table 1) fit to juvenile white sturgeon released in the Kootenai River from 1992 to 2004.

				Noncovariate models		All models	
Model	Model notation	Parameters	QAIC	ΔQAIC	QAIC weights	ΔQAIC	QAIC weights
1	$\varphi(t), p(t)$	26	18 395.3	766.7	0.000	1 012.1	0.000
2	$\varphi(a2\text{-}c/c), p(t)$	15	18 194.4	565.8	0.000	811.3	0.000
3	$\varphi(a2\text{-}t/c), p(t)$	24	17 630.7	2.1	0.210	247.6	0.000
4	$\varphi(a2\text{-}t/t), p(t)$	34	17 643.0	14.4	0.000	259.8	0.000
5	$\varphi(a3\text{-}t/c/c), p(t)$	25	17 631.2	2.6	0.168	248.0	0.000
6	$\varphi(a3\text{-}t/t/c), p(t)$	33	17 628.6	**0.0**	**0.611**	245.4	0.000
7	$\varphi(a3\text{-}t/t/t), p(t)$	44	17 644.8	16.2	0.000	261.6	0.000
8	$\varphi(a3\text{-}t/t/c), p(a2\text{-}t/t)$	42	17 636.7	8.1	0.010	253.6	0.000
9	$\varphi(a3\text{-}t/t/t), p(a2\text{-}t/t)$	53	17 654.5	25.9	0.000	271.3	0.000
10	$\varphi(a3\text{-}l + t/t/c), p(t)$	34	17 422.4	—	—	39.3	0.000
11	$\varphi(a3\text{-}l \times t/t/c), p(t)$	43	17 383.2	—	—	**0.0**	**1.000**

Note: Values of quasi-Akaike information criterion (QAIC) for all noncovariate models (Models 1–9) and length covariate models (Models 10–11) were computed using the overdispersion parameter ($\hat{c}$ = 1.15) estimated for the global noncovariate model (Model 9). The QAIC results of the best-fitting noncovariate and covariate models are noted in bold.

Table 5. Estimates of survival rate and recapture probability (95% confidence intervals in parentheses) for the best-fitting noncovariate model (Model 6) and length covariate model (Model 11) for juvenile white sturgeon released into the Kootenai River from 1992 to 2004.

	Model 6			Model 11	
Year	Recapture probability	Age-1 survival	Age-2 survival	Age-1 survival	Age-2 survival
1992	—	0.70 (0.57, 0.86)	—	0.75 (0.57, 0.87)	—
1993	0.01 (0.00, 0.06)	—	Set to 1.0	—	Set to 1.0
1994	—	0.61 (0.48, 0.81)	—	0.64 (0.36, 0.85)	—
1995	0.17 (0.11, 0.24)	—	1.00 (1.00, 1.00)	—	1.00 (1.00, 1.00)
1996	0.27 (0.20, 0.36)	—	—	—	—
1997	0.14 (0.08, 0.20)	0.73 (0.60, 0.91)	—	0.63 (0.49, 0.75)	—
1998	0.12 (0.09, 0.15)	0.84 (0.57, 1.00)	0.63 (0.50, 0.79)	0.45 (0.03, 0.95)	0.66 (0.52, 0.79)
1999	0.12 (0.10, 0.15)	0.30 (0.21, 0.42)	0.89 (0.54, 1.00)	0.26 (0.17, 0.36)	0.93 (0.56, 1.00)
2000	0.15 (0.12, 0.17)	0.49 (0.43, 0.60)	1.00 (0.71, 1.00)	0.46 (0.39, 0.53)	1.00 (1.00, 1.00)
2001	0.14 (0.12, 0.16)	0.35 (0.30, 0.40)	1.00 (1.00, 1.00)	0.23 (0.19, 0.27)	1.00 (1.00, 1.00)
2002	0.07 (0.06, 0.09)	0.28 (0.21, 0.39)	1.00 (1.00, 1.00)	0.21 (0.15, 0.30)	1.00 (1.00, 1.00)
2003	0.05 (0.04, 0.06)	0.01 (0.00, 0.02)	0.76 (0.52, 1.00)	0.01 (0.00, 0.03)	0.76 (0.42, 0.93)
2004	0.06 (0.05, 0.07)	0.16 (0.12, 0.20)	0.47 (0.09, 1.00)	0.17 (0.14, 0.22)	0.48 (0.05, 0.94)
2005	0.04 (0.03, 0.04)	—	—	—	—

Note: Age-1 survival estimates for Model 11 were based on average-sized juvenile white sturgeon (mean fork length = 20 cm, standard deviation = 5 cm). Recapture probabilities and age-3+ survival estimates for Model 11 were identical to those for Model 6.

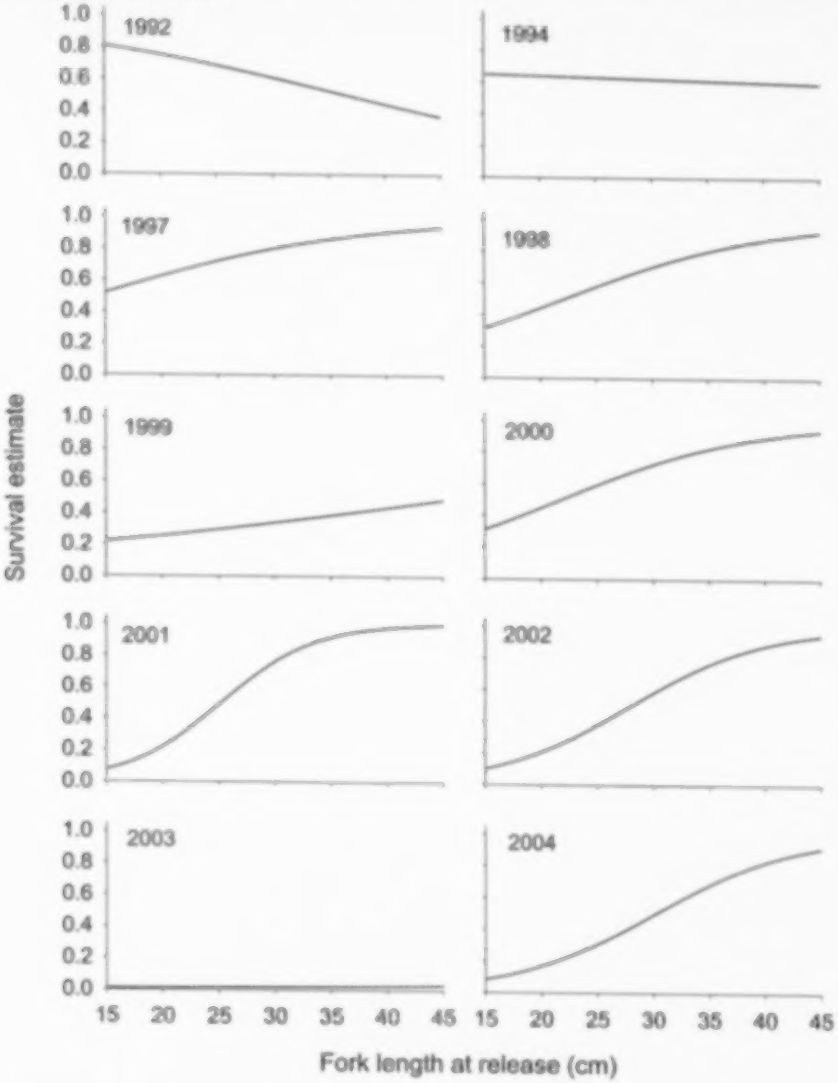
white sturgeon behavior and survival. Temperature data at Porthill was unavailable for 1997 and 2002. To supplement missing data for 2002, we used daily maximum temperatures collected at the Kootenai Tribal Sturgeon Hatchery. A comparison of August temperatures at the two locations across 3 years with overlapping data indicated that temperatures were very similar, with Porthill data averaging 0.2 °C cooler.

As an annual measure of Kootenai River white sturgeon abundance, we summed the total number of new releases (excluding lake releases) and the estimated number of fish that survived from previous years. The latter was estimated using the annual survival rates derived from the best-fitting noncovariate model (Model 6). We used age-1 survival estimates from both Model 6 and the best-fitting covariate model (Model 11) as dependent variables in regressions with total abundance and environmental variables. The latter comparison was used to test whether the decline in age-1 survival was related to total abundance or other environmental factors after removing the effect of size-at-release. Assumptions of the linear regressions were tested using graphical methods, the Shapiro–Wilk test for normality, and the Durbin–Watson test for autocorrelation.

Results

A total of 119 768 hatchery-reared juveniles were released between 1992 and 2006 (Table 2). These included 39 275 PIT-tagged fish released in the river, 14 306 released into Kootenay Lake, and 66 187 fish released without PIT tags. Excluding lake releases, unmarked fish, and individuals with missing release information, the number of PIT-tagged releases from 1992 to 2005 totalled 36 765 (Table 3). Initial releases were relatively small but increased substantially following development of rearing facilities at the BC hatchery

Fig. 3. Relationships between fork length-at-release (cm) and age-1 survival as estimated from the best-fitting covariate model (Model 11). No fish were released in 1993, 1995, and 1996.



and upgrades to the Kootenai Tribal Hatchery in 1998 and 1999. Historically, individual fish size-at-release varied widely, ranging from 7 to 64 cm (mean = 20 cm; standard deviation, SD = 4 cm). More recently, smaller fish were released to maximize hatchery production (Fig. 2).

A total of 2938 PIT-tagged juveniles were recaptured between 1992 through 2006. Recaptures totaled 2350 after removing within-year recaptures and a small number of untraceable fish (Table 3).

Survival rates

Fits of the noncovariate capture–recapture models (Models 1–9) provided strong evidence of both age-specific and year-specific differences in survival rates of juvenile white sturgeon in the Kootenai River. The best-fitting model based on QAIC was Model 6 (Table 4), which specified year-specific survival rates for the first two age classes (age-1 and age-2), constant survival rates for the third age class (age-3+), but no age structure for recapture probabilities (Table 1). As model complexity increased, by far the largest improvement in fit (i.e., reduction in QAIC) resulted from the addition of year-specific survival rates for age-1 fish (Table 4). For example, ΔQAIC declined from 565.8 for Model 2 to 2.1 for Model 3. In contrast, the addition of year-specific survival for age-2 fish resulted in only a slight improvement in model fit (Model 6, ΔQAIC = 0). Model 5, which assumed constant survival across years for age-2 fish, was also a strong competing model (ΔQAIC = 2.6). In addition, there was little evidence that survival rates of age-3+ fish varied across years (Model 7, ΔQAIC = 16.2). Collectively, these results indicate (*i*) very strong empirical support that age-1 survival rates were distinctly time varying; (*ii*) comparatively weak evidence that age-2 survival rates either differed from older age classes or were distinctly time varying; and (*iii*) moderate support that survival rates of age-3+ fish were essentially constant across years.

Parameter estimates for the best model (Model 6) indicated that age-1 survival rates declined markedly over time, ranging from a high of 0.84 in release year 1998 to only 0.01 in 2003 (mean = 0.45) (Table 5). Survival estimates for age-2 fish were generally higher and less variable, ranging from 0.48 to 1.0 (mean = 0.84) (Table 5). In contrast, age-3+ survival was estimated to be 1.0 (95% confidence interval, CI = (0.97, 1.00)) across all years. Clearly, a survival rate of 1.0 (i.e., 100%) for age-3+ fish is unrealistically high. Nevertheless, this result suggests that age-3+ survival was consistently high across years but was statistically indistinguishable from 1.0 because of limited data and sampling error.

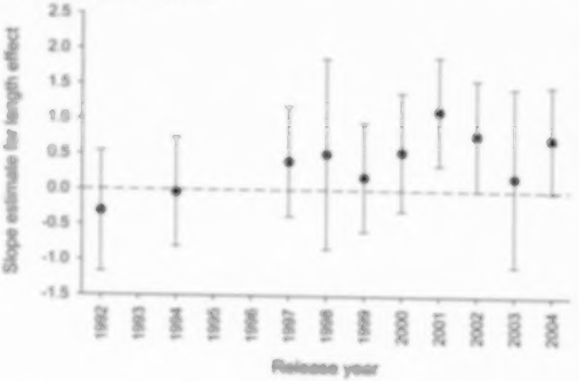
Survival estimates for age-1 fish were reasonably robust to assumptions regarding age structure. Across years, estimates for Model 6 were similar to, and highly correlated with, those for the top two competing models (r = 0.97 for Model 3 with no age-2 class; r = 0.97 for Model 5 with constant survival for age-2 fish), with absolute differences in age-1 survival estimates averaging less than 0.05 in both cases. The most obvious difference in age-1 survival estimates occurred for release year 1997, where survival was estimated to be 0.73 for Model 6 compared with 0.54 and 0.56 for Models 3 and 5, respectively.

Finally, there was little empirical support that recapture rates of age-1 fish differed substantially from those of older fish (Model 6 versus Model 8, ΔQAIC = 8.1; Table 4). For Model 8, estimates of recapture rates of age-1 fish were 26% lower on average than those for age-2+ fish, which is consistent with the hypothesis that young juveniles may be less vulnerable to recapture because of size-selective gear effects. However, there was considerable overlap among year-specific confidence intervals for the recaptures rates of each age group, and overall, little statistical support for such differences (ΔQAIC = 8.1). Importantly, age-1 survival estimates for Model 8 were again similar to those for Model 6 (r = 0.97; average absolute difference = 0.05).

Effect of size-at-release

The addition of length covariates to Model 6 greatly improved the model fit, suggesting that age-1 survival rates were strongly influenced by the length-at-release of individual fish. The best-fitting length covariate model based on QAIC was Model 11 (Table 4). This model incorporated a year-specific length effect for age-1 survival, indicating that the functional form (slope) of the length effect varied across years. Indeed, parameter estimates for Model 11 depicted considerable variation across years in the relationship between length and age-1 survival rates (Fig. 3). The influence of length is reflected by estimated slopes between length and logit(survival) (Fig. 4). Although slope estimates were imprecise, the direction of the length effect was consistently positive beginning with the 1997 release year (Figs. 3 and 4). This positive effect of fish length on first year survival tended to increase over time and was highest for 2001 releases.

Fig. 4. Slope estimates (Model 11) for the effect of fork length-at-release (cm) on logit(survival) of age-1 fish for release years 1992–2004. Error bars represent approximate 95% confidence intervals. Values greater than zero (above broken line) indicate a positive effect of length on survival.



Annual estimates of age-1 survival for Model 11 are reported in terms of the average-sized fish across release years (mean = 20 cm, SD = 5 cm) (Table 5). These estimates were similar to the baseline survival estimates provided by Model 6 and showed a steady decline across years from a high a 0.75 in 1992 to a low of 0.01 in 2003 (mean = 0.38). The most notable difference between age-1 survival estimates was for 1998 releases (0.84 for Model 6 and 0.45 for Model 11). The estimated survival rate for 1998 was lower under Model 11 (the length covariate model) because this estimate was for an average-sized fish across years (20 cm), whereas the actual average length of 1998 releases was considerably greater (36 cm) (Fig. 2). In general, however, the similar declining patterns in age-1 survival estimates for Models 6 and 11 suggest that while length-at-release had important effects on survival, particularly in recent years, other factors were largely responsible for the declining trend in first year survival rates.

Comparisons of length-at-release distributions between fish that were recaptured and not recaptured generally corroborated the results from the length covariate models. That is, fish released at a larger size were more likely to survive and be recaptured for all releases since 1997 (Table 6). Mean length-at-release was significantly higher for fish that were recaptured versus not recaptured in five out of eight comparisons since 1997 (Wilcoxon test: $P < 0.05$). The largest positive differences in mean length (recaptured minus not recaptured) occurred for fish released in 2001 (difference = 2.5 cm) and 2002 (5.6 cm) (Fig. 5).

Effect of environmental conditions and fish abundance

Between 1992 and 2005, median annual river discharge in the Kootenai River at Porthill, Idaho, ranged from a low of 199 $m^3 \cdot s^{-1}$ in 2001 to a high of 561 $m^3 \cdot s^{-1}$ in 1996 (Fig. 6*a*). Relatively high flood events occurred in 1996 and 1997, with peak discharges exceeding 1500 $m^3 \cdot s^{-1}$ in both years. Median daily maximum stream temperatures during August ranged from 15.5 to 20.5 °C across years (median all years =

Table 6. Summary of Wilcoxon rank sum tests for comparisons between length-at-release distributions for juvenile sturgeon released but not recaptured and those recaptured.

	Mean fork length (cm)			Wilcoxon test		
Release year	Not recaptured	Recaptured	Difference	df	*W*	*P* value
1992	24.6	23.2	–1.4	115	–0.3	0.755
1994	41.8	41.2	–0.6	118	2.2	0.030
1997	23.9	25.3	1.4	1 948	–4.3	<0.001
1998	35.2	36.6	1.4	94	–1.3	0.209
1999	24.2	27.1	2.9	331	–0.4	0.664
2000	21.4	21.9	0.5	2 184	–2.5	0.013
2001	21.9	24.4	2.5	6 011	–12.8	<0.001
2002	21.0	26.6	5.6	3 791	–8.8	<0.001
2003	18.6	19.3	0.6	8 378	–0.5	0.625
2004	19.1	20.3	1.1	12 537	–4.1	<0.001

Note: Difference refers to the difference between means (recapture – release); df refers to degrees of freedom.

Fig. 5. Distributions of length-at-release for juvenile sturgeon not recaptured (black bars) and recaptured (grey bars) for example release years (*a*) 2001 and (*b*) 2002.

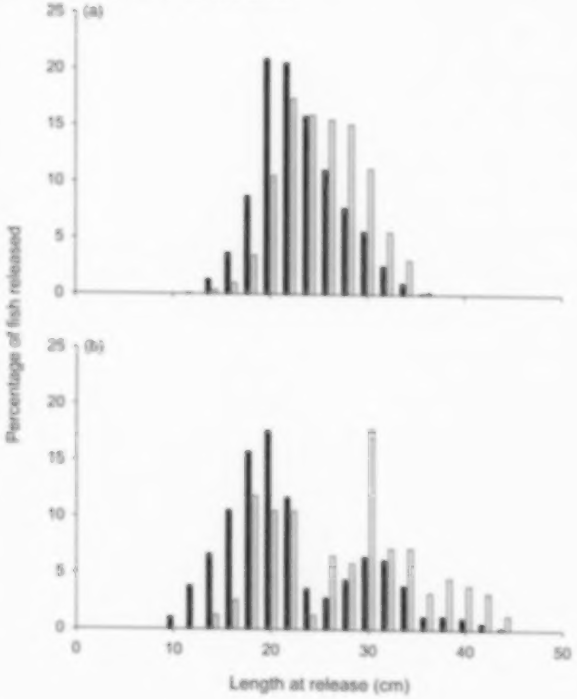
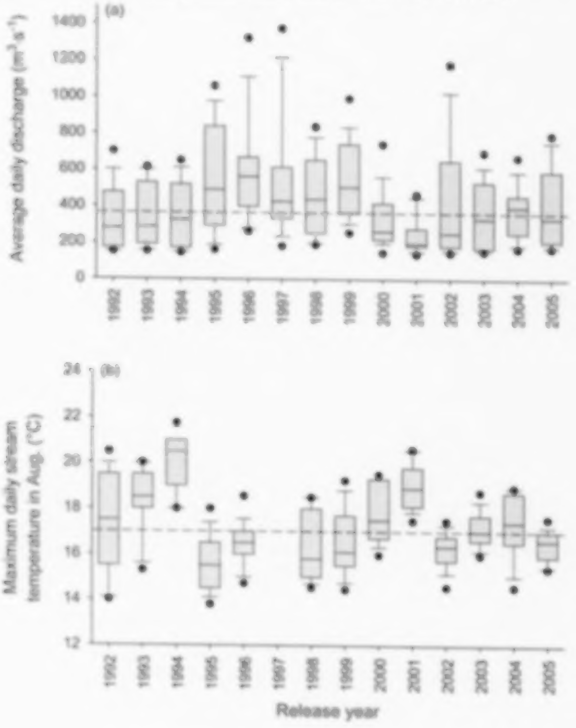


Fig. 6. Distributions of (*a*) average daily discharge ($m^3 \cdot s^{-1}$) and (*b*) maximum daily river temperatures (°C) during August in the Kootenai River near Porthill, Idaho, for release years 1992–2005. Box plots show medians (horizontal lines), 25th–75th percentiles (boxes), 10th–90th percentiles (whiskers), and 5th–95th percentiles (dots). The broken horizontal line represents the median discharge across all years. Temperature data was unavailable for 1997.

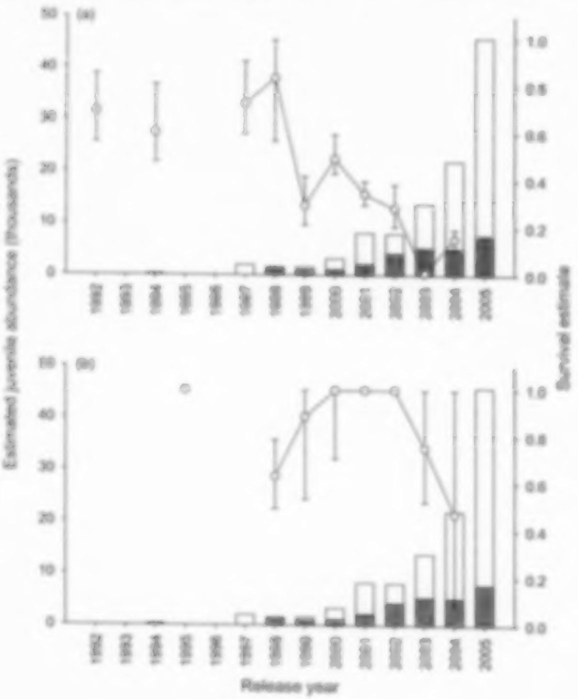


17.0 °C) (Fig. 6*b*), with the warmest temperatures occurring in 1994 and 2001.

No significant relationships were observed between estimates of age-1 survival rate (Model 6 and Model 11; Table 5) and annual averages of environmental conditions (discharge, maximum discharge, and maximum daily temperatures) (linear regression: $P > 0.05$). These factors explained little of the annual variation in age-1 survival rates, with R^2 values ranging from 0.02 to 0.04.

In contrast, there was clear association between estimates of age-1 survival and fish abundance. Recent declines in survival coincided with increases in both hatchery releases and estimates of age-2+ abundance (Fig. 7*a*). Abundances of age-2+ fish, which were estimated via survival estimates from Model 6, increased steadily from 82 fish in 1993 to 7897 fish in 2005. A linear regression of total juvenile abundance versus age-1 survival estimated from the best-fitting

Fig. 7. Estimated abundance (total releases + residual population) of hatchery-reared juvenile white sturgeon compared with (*a*) age-1 survival rates and (*b*) age-2 survival rates for release years 1992–2005. New releases, residual population, and survival rates are denoted by open bars, solid bars, and circles, respectively. Survival estimates were derived from the best-fitting noncovariate (Model 6).



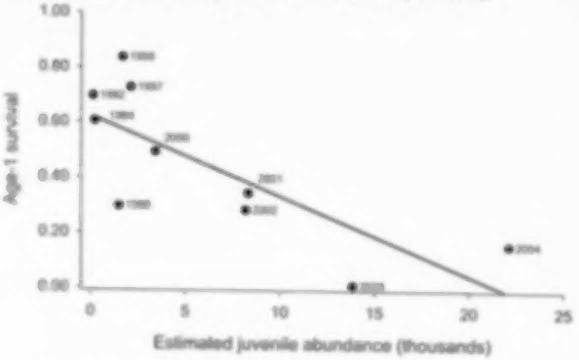
noncovariate model (Model 6) explained 59% of the variation in age-1 survival rates (Fig. 8; $P = 0.01$). Similarly, age-1 survival rates estimated using the best fitting covariate model (Model 11) were also negatively related to total juvenile abundance ($R^2 = 0.56$, $P = 0.01$). There was little evidence of a relationship between juvenile abundance and age-2 survival estimates (Fig. 7*b*).

Discussion

Survival of hatchery-reared juvenile white sturgeon declined in the first year following release into the Kootenai River in an apparent response to the cumulative effects of size- and density-dependent factors since the first hatchery releases in 1992. Prior to 1999, first year survival following release was relatively high, averaging 72%, and was not strongly related to fish size. First year survival of more recent releases declined to just 1%–49% per year (mean = 27%), accompanied by lower survival among the smaller fish within most release groups. In contrast, survival of age-2 fish did not exhibit a consistent trend over time, with estimates ranging from 48% to 100% (mean = 84%). After their second year in the wild, survival remained high (close to 100%) throughout the study period.

Poor first year survival of juvenile white sturgeon coincided with hatchery practices to release greater numbers of small fish beginning in 2002. Hatchery production capacity is limited by juvenile rearing space. Releasing smaller, younger fish allowed the hatchery to produce more juveniles by avoiding seasonal space limitations caused by extended rearing of multiple overlapping brood years. The decision to release more and smaller fish was precipitated by projected demographic bottlenecks in wild abundance (Paragamian et al. 2005) and high rates of survival observed among initial release groups (Ireland et al. 2002*b*). It appears that this decision did not produce the expected demographic benefit because of declines in survival of smaller fish concurrent with increased production.

Fig. 8. Relationship between annual estimates of juvenile sturgeon abundance and age-1 survival rates. Survival estimates were derived from the best-fitting noncovariate model (Model 6).



Our results suggest that density-dependent factors may be limiting survival of hatchery-reared juvenile white sturgeon in the Kootenai River. The decline in first year survival rates of the small white sturgeon in recent release groups coincided with increasing annual release numbers and increasing abundance of juvenile white sturgeon from previous releases. In contrast, there was little evidence to suggest that annual variation in environmental conditions such as discharge and river temperature substantially influenced survival of hatchery-reared sturgeon. This is the first reported evidence for density-dependent population dynamic processes in North American sturgeon of which we are aware. Density-dependent mortality appears to be concentrated among smaller juveniles (e.g., <25 cm in fork length) during their first year after release. Larger, older juveniles do not currently appear subject to the same limiting factors.

Collectively, these results appear to support a combined effect of both size-at-release and release number on survival of Kootenai River white sturgeon during their first year at large. Recent management decisions to release greater numbers of hatchery fish coincided with a reduction in average size-at-release, potentially confounding any distinct effects of density- and size-related mortality. Yet our findings provide evidence in support of both processes. Capture–recapture models that included a length covariate were heavily favored over the other models in terms of QAIC, suggesting strong empirical support for an effect of length-at-release on first year survival rates. In addition, for a fixed length-at-release of 20 cm (i.e., after accounting for annual differences in mean length-at-release), age-1 survival estimates still showed a strong declining trend in recent years (Model 11) and a significant negative relationship with juvenile abundance ($R^2 = 0.56$, $P = 0.01$), indicating that much of the variation in first year survival was driven by other factors such as stocking densities or unknown environmental conditions. Thus, although the relative impacts of stocking density and size-at-release on first year survival remain uncertain, we contend that both factors should be considered carefully in future management and monitoring strategies.

The biological mechanisms driving the apparent density-related declines in first year survival of Kootenai River white sturgeon are unknown but might include competition or predation. Recent stocking levels of small sturgeon may have exceeded the current rearing capacity of the Kootenai River for this life stage. White sturgeon are also highly piscivorous, and the larger individuals from previous releases might be cannibalizing the smaller fish from more recent releases. Productivity of the Kootenai River has declined from historic levels, and food limitations are a real possibility. Lake Koocanusa, formed by construction of Libby Dam in 1972, is a nutrient sink, trapping nearly 63% of available nitrates and 25% of the phosphates destined for the lower Kootenai River (Woods 1982; Snyder and Minshall 1996). A recent energy budget developed for the Kootenai River indicated that autotrophic and detrital energy inputs were insufficient to sustain the estimated fish biomass downstream of Libby Dam (Snyder and Minshall 2005). In addition, estimated growth rates and condition factors of hatchery-reared juvenile white sturgeon in the Kootenai River were generally lower than averages for other populations (Ireland et al. 2002*b*).

Density-dependent rearing limitations among small juvenile white sturgeon in the Kootenai River may represent a critical second recruitment bottleneck. Other research has identified a severe bottleneck at the egg incubation stage (Paragamian and Wakkinen 2002). Significant numbers of fish are observed to spawn each year, and viability of embryos has been confirmed, but most spawning currently occurs over an unstable sand and silt substrate that is not conducive to survival (Paragamian et al. 2001; Anders et al. 2002; Kock et al. 2006). A density-related rearing limitation for young juveniles (e.g., age-1 fish) would be a second limiting life stage. Although this potential limitation was identified using hatchery fish, it is possible that a similar problem contributed to the historical decline in wild recruitment in the absence of hatchery fish. This second bottleneck would clearly compound the difficulty of restoring substantial natural recruitment. This finding highlights the potential utility of hatchery fish as surrogates for providing critical empirical information on survival bottlenecks and habitat limitations on natural production. However, competition with large numbers of hatchery fish could also reduce survival of wild sturgeon and complicate evaluations of other recovery measures.

The results from this study have important implications for the strategic goals of the aquaculture conservation program, recovery of white sturgeon in the Kootenai River, and other sturgeon populations at risk. Continued releases of large numbers of small-sized fish may severely limit survival of juvenile white sturgeon during the first year following release. Given the high estimated survival rates for age-2+ fish, management actions that prioritize the release of fewer, larger-sized fish will likely improve first year survival rates and subsequent recruitment to the spawning-age population. We recommend that (*i*) stocking numbers should be based on habitat suitability, expected survival, and available habitat; (*ii*) releases of hatchery white sturgeon should be contingent on abundance of wild white sturgeon of a similar year class as determined through research monitoring and evaluation; and (*iii*) a total hatchery population goal be developed based on stocking numbers, survival rates, and expected hatchery contribution to the total white sturgeon population. Consistent with an adaptive management strategy, the hatchery program has already implemented strategies to increase body size and reduce numbers of releases as suggested from this study.

While conservation aquaculture is a vital component to successful restoration of white sturgeon in the Kootenai River, it is only one aspect of an integrated ecosystem restoration program designed to address limiting factors for reproduction and recruitment. A variety of other actions have been implemented to improve conditions for recruitment in the wild, including flow and temperature management, river and tributary habitat restoration, nutrient augmentation, and re-establishment of native fish populations. Whether these efforts and the Kootenai hatchery program can effectively propagate white sturgeon through the next generation will depend on the ability of managers to anticipate, avoid, and adapt to new problems while pioneering a conservation recovery strategy with very limited room for error.

Acknowledgements

This work reflects the dedicated efforts of many people involved in Kootenai white sturgeon recovery planning, hatchery operation, and population monitoring over the years. The mark–recapture database central to this analysis was developed and maintained by Virginia Wakkinen of the Idaho Department of Fish and Game. Funding for completion of this project was provided by the Bonneville Power Administration through the Kootenai Tribe of Idaho.

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Recent shifts in the crustacean zooplankton community of Lake Huron

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Abstract: Cladoceran populations in the open waters of Lake Huron declined abruptly in 2003 and have since remained at historically low levels. The two dominant cladocerans, *Daphnia mendotae* and *Bosmina longirostris*, have been nearly extirpated from the northern region of the lake and are present in only slightly greater numbers in the south. Average nonpredatory cladoceran biomass in the lake has declined over 90% between 1998–2002 and 2003–2006. In addition, historically unprecedented declines in cyclopoid copepods were seen in the lake in 2005. These changes have occurred against the backdrop of declining nutrient levels in the lake and have coincided closely with declines in the amphipod *Diporeia*. We speculate that a combination of reduced primary production in the open waters and intensified planktivory due to the continuing disappearance of *Diporeia* has accounted for the losses in crustacean biomass seen in recent years.

Résumé : Les populations de cladocères dans les eaux du large du lac Huron ont décru abruptement en 2003 et sont demeurées à des densités historiquement basses. Les deux cladocères dominants, *Daphnia mendotae* et *Bosmina longirostris*, ont été presque extirpés de la région nordique du lac et sont présents en nombres à peine plus élevés dans le sud. La biomasse moyenne des cladocères non prédateurs dans le lac a diminué de plus de 90 % entre 1998–2002 et 2003–2006. De plus, il s'est produit des déclins jamais vus chez les copépodes cyclopoïdes dans le lac en 2005. Ces changements sont survenus dans des conditions de diminution des nutriments dans le lac et ont coïncidé étroitement avec les déclins de l'amphipode *Diporeia*. Nous avançons l'hypothèse selon laquelle une combinaison de production primaire réduite dans les eaux libres et de planctonophagie accrue causée par la disparition persistante de *Diporeia* explique les pertes de biomasses des crustacés observées ces dernières années.

[Traduit par la Rédaction]

Introduction

Lake Huron, the second largest of the Laurentian Great Lakes in terms of surface area (Beeton 1984) and the fourth largest in the world (Beeton and Saylor 1995), has experienced a number of major shifts in its biological communities during the last century. The earliest indications of major ecosystem change were apparent in the lake's fish community. Originally supporting a diverse community of cold-water and cool-water fish, by the mid 1960s the native top predator, lake trout (*Salvelinus fontinalis*), had nearly disappeared, and four species of deepwater ciscoes (*Coregonus* spp.) were completely extirpated, while populations of whitefish (*Coregonus clupeaformis*) and cisco (*Coregonus artedi*) were reduced to extremely low levels (Berst and Spangler 1973; Bailey and Smith 1981). These changes occurred because of a combination of overfishing, habitat deterioration, and interactions with exotic species that entered the lake during the early to mid-20th century, in particular sea lamprey (*Petromyzon marinus*), rainbow smelt (*Osmerus mordax*), and alewife (*Alosa pseudoharengus*) (Ebner et al. 1995). Accordingly, for most of the last 50 years, the fish community in Lake Huron has been largely dominated by exotics; top predator populations have been sustained through large scale stocking of salmonines, while rainbow smelt and alewives have continued to dominate the prey fish community, along with the native bloater (*Coregonus hoyi*) (Ebner et al. 1995).

The lower food web of the lake has also been subject to substantial perturbation. The exotic predatory cladoceran *Bythotrephes longimanus* was first detected in the lake in 1984 (Bur et al. 1986) and has been implicated in marked shifts in zooplankton community composition, including reductions in the populations of several cladoceran species and a resultant decrease in species richness (Barbiero and Tuchman 2004). The zebra mussel *Dreissena polymorpha* first became established in the lake by 1989 (Griffiths et al. 1991), and impacts were soon thereafter seen in shallower portions of the Lake Huron ecosystem (Fahnenstiel et al. 1995). Substantial declines in populations of the amphipod *Diporeia*, an important fish food item, were also noted in Lake Huron

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by 1999 (Barbiero and Tuchman 2002), as had been seen in Lake Michigan (Nalepa et al. 1998). Meanwhile, reductions in phosphorus loading mandated by the Great Lakes Water Quality Agreement have resulted in a drop in loads to Lake Huron from 5.1 million kg·year^{-1} in 1978 to 4.3 million kg·year^{-1} by 1981, and loading has remained below target levels in all but a few years through the late 1990s (Beeton et al. 1999).

It appears that the lake is once more in the midst of a period of considerable ecosystem change, as evidenced by a number of large and abrupt shifts in several populations that took place around 2003. Populations of adult alewife, which had been one of the main prey fish in the lake since the 1960s, collapsed in 2003, and this species has since been reduced to unprecedentedly low levels in the main basin (Riley et al. 2008). The cause for this collapse is currently under investigation. Coincident with this collapse has been a dramatic upsurge in natural reproduction of the native lake trout (Riley et al. 2007). In addition, populations of the deep-living dreissenid mussel *Dreissena bugensis*, which had been nearly absent from the lake in 2000, had increased to densities approaching 1500 individuals·m^{-2} in the 31–50 m depth stratum in 2003, effectively extending the range of dreissenid colonization to deeper areas of the lake (Nalepa et al. 2007).

A critical component of the food web that has received very little attention so far in the midst of these recent ecosystem perturbations is the crustacean zooplankton community. In this paper we report on recent dramatic changes that have occurred in the zooplankton community of Lake Huron since 2003, utilizing annual zooplankton survey information generated from the US Environmental Protection Agency's (USEPA) Great Lakes National Program Office's (GLNPO) regular spring and midsummer cruises. We evaluate these data in the context of associated nutrient and benthos data.

Materials and methods

Sampling

Data were drawn from USEPA GLNPO offshore monitoring cruises conducted between 1984 and 2006. A total of 20 stations were visited for zooplankton and nutrient sampling, although not all stations were visited during all years (Fig. 1). Benthos samples were taken at seven of these sites, as well as an additional eighth site, to estimate *Diporeia* populations. Past analyses have shown that differences exist both in the biology and the chemistry of different regions of the lake, which largely correspond to differences in lake basin morphometry. Therefore, for most analyses, stations were allocated to either the deeper northern or the shallower southern portion of the lake (as shown in Fig. 1).

Sampling surveys were conducted during the spring isothermal period and summer stratified period. Spring (April) surveys were conducted as early as possible after ice-out to provide estimates of initial growing season concentrations of nutrients and took place in all years between 28 March and 30 April. Water temperatures at the time of the spring surveys were relatively constant, ranging between 0.9 and 2.9 °C, with the exception of 2003 when temperatures were 0.4–0.5 °C. Summer (August) surveys were conducted during the period of stable thermal stratification and took place between 3 August and 29 August. From 1984 to 1991, multiple sampling runs were often undertaken for one or both of the seasonal surveys. While sometimes separated by several weeks, no consistent differences were found in the data generated by multiple runs, so data from all runs were included in annual survey averages.

At each station, samples for nutrients were taken at discrete depths throughout the entire water column with Niskin bottles mounted on a SeaBird Carousel Water Sampler. For the present study, only samples from the isothermal upper ($\leq$12 m) water column were used. Since shipboard operations were conducted on a 24 h basis, Secchi depths were measured at those stations visited between 1 h after sunrise and 1 h before sunset.

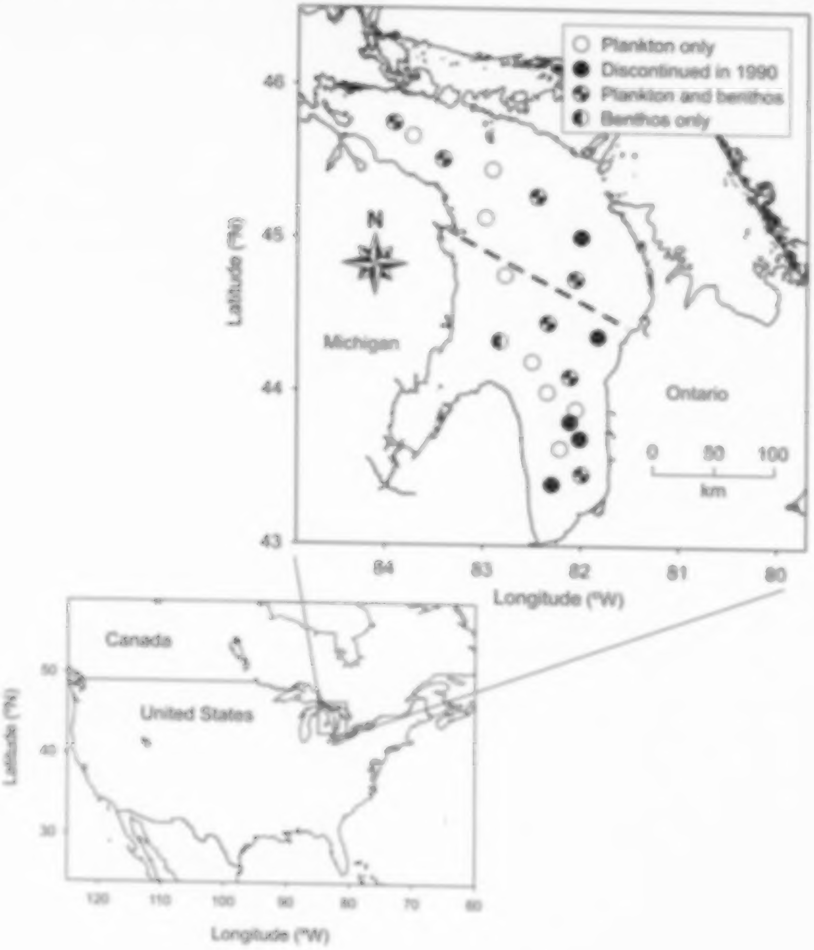
During the course of the monitoring program, zooplankton sampling methods have changed somewhat. From 1984 to 1990, zooplankton were collected with tows taken from depths of both 20 and 2 m above the bottom using a 0.5 m diameter, 64 µm mesh conical net (D:L = 1:3) equipped with a flow meter. The deeper tows were discontinued in the early 1990s. Beginning in 1997, deep tows were again taken, albeit with a maximum depth of 100 m and using a coarser mesh (153 µm). Neither net size nor shape changed. Since 20 m tows would likely miss a large portion of the zooplankton community, only data from deeper tows will be presented here. Triplicate samples for *Diporeia* population estimates were collected using a ponar grab during August surveys from 1997 to 2006.

Analytical methods

Samples for soluble Si were filtered in the field through 0.45 µm Sartorius filters and stored at 4 °C, and samples for total phosphorus (TP) were preserved with H_2SO_4 for later analysis in the lab. TP was measured on a Lachat QuikChem AE autoanalyzer by the ascorbic acid method after acid persulfate digestion (American Public Health Association 1985). Soluble Si was determined as SiO_2 by the molybdate method on a Lachat QuikChem AE autoanalyzer (American Public Health Association 1985). Prior to 1991, chemical analyses were conducted onboard ship; after this date, analyses were performed in a land-based laboratory. Internal comparisons have shown no difference and confirmed the comparability of data from the two periods (G. Warren, USEPA GLNPO, 77 W. Jackson Blvd., Chicago, Illinois, unpublished data). Prior to 1999, chlorophyll *a*, corrected for pheophytin, was determined on a Turner Designs 10-AU fluorometer after extraction in 90% acetone. After 1999, chlorophyll *a* was measured using narrow band optical filters, following the method of Welschmeyer (1994).

After collection, zooplankton samples were narcotized with soda water and preserved with 5% formalin solution. Samples were split in the laboratory using a Folsom plankton splitter, and four stratified aliquots were examined per sample using a stereoscopic microscope such that rare species were enumerated from the less dilute aliquots. On average, about 600 individuals were identified per sample. Length measurements were made on the first 20 individuals of each species encountered per sample, and biomass (as dry weight) was calculated using length–weight relationships de-

Fig. 1. Map of Lake Huron showing locations of sampling stations. The broken line indicates division between northern and southern stations.



rived from the literature (Downing and Rigler 1984). Prior to 1997, length measurements were not taken on deeper tow samples. Therefore, biomass for those tows was estimated using species-specific average length measurements made on individuals from 20 m tows collected during the same cruise. Since within many species larger individuals might be expected to occupy deeper portions of the water column during the day, a low bias might be introduced by this approach.

Data analysis

For water quality variables, station averages were first calculated using discrete samples collected from depths ≤ 12 m. This ensured that values were restricted to the upper isothermal portion of the water column. Annual, basin-wide averages were then calculated from these station averages for each cruise (i.e., April and August). All stations visited during all cruises during a given seasonal survey within a basin were thus treated as replicates.

For zooplankton, basin-wide average densities and biomass were calculated for each cruise, using all stations visited. Unlike water quality variables, zooplankton samples were taken on only one cruise per season, with the sole exception of 1984 when two summer cruises were undertaken. Data from these two cruises were pooled for most analyses, but were kept separate for multivariate analyses to provide an indication of sampling variability. For the 1998–2006 data, basin-averaged biomass by size category was also calculated for all crustaceans. For each species, length measurements from each station in the basin were increased proportionately to the species density at that station relative to the basin-wide average using a random sampling routine, such that the total number of length measurements equaled the basin-wide average density for that species. Biomass was then calculated for each length measurement and summed by size category. As noted, length measurements were not taken on deep tow samples during the previous years, precluding such analyses for those years.

Interannual shifts in crustacean community composition were quantitatively explored using cluster analysis and nonmetric multidimensional scaling (MDS). To prevent basin-level differences in biomass from obscuring the directionality of year-to-year changes in community composition, whole-lake averages were used for multivariate analyses. Similar results were obtained when separate basin-averaged analyses were conducted. Years with similar species composition were determined with hierarchical cluster analysis, using group averages, for which biomass data were standardized. A similarity value of 0.75 was used to delineate cluster groups. MDS is an ordination technique that relies upon rank similarities and thus is insensitive to nonnormality in the data and does not assume linear relationships among variables (Clarke 1993). A Bray–Curtis similarity matrix using square-root transformed biomass values served as input for the analysis. Relationships between individual species and ordination axis scores were determined using Spearman rank correlation analysis. Owing to the relatively large number of variables involved in the analysis, a conservative significance level of $\alpha = 0.01$ was used. To confirm the results of cluster analysis, an analysis of similarities (ANOSIM), a multivariate analogue of analysis of variance (ANOVA), was used (Clarke and Green 1988), with cluster-derived time periods as the main factor levels. This analysis is based on a nonparametric permutation procedure in which a Bray–Curtis similarity matrix derived from all possible combinations of samples is analyzed as for a one-way ANOVA. The resulting statistic, *R*, theoretically ranges from –1 to 1 and is associated with a probability derived from the simulated test statistic distribution expected under the null hypothesis. Multivariate analyses were performed using PRIMER version 5.2.9 (Primer-E Ltd., Plymouth Marine Laboratory, Plymouth, UK, www.primer-e.com).

Differences in biomass values between the different periods were assessed using a generalized linear model (GLM), with cluster-derived time periods the main factor effect, year nested within period, and stations serving as replicates within each year. Biomass data was log-transformed prior to analysis. The two basins were not analyzed separately unless a significant basin × period interaction was found, in which case separate analyses were conducted for each basin. Where a significant main factor effect was found, a Tukey–Kramer pairwise multiple comparison test was used to determine differences between specific time periods. Significance was assessed at $\alpha = 0.05$. These analyses were carried out using SAS (SAS Institute Inc., Cary, North Carolina).

Overall trends in environmental variables were assessed using the nonparametric Mann–Kendall test. Separate analyses were carried out for each variable–basin combination. Given the large number of observations, the normal approximation was used to assess significance at $\alpha = 0.05$. To assess differences in environmental variables during the more recent (1998–2006) portion of the data set, ANOVA analyses were conducted with the two latest time periods (identified by cluster analysis) serving as main factor effects, years nested within time period, and station treated as replicates. Separate analyses were carried out for each variable–basin combination. Significance was assessed at $\alpha = 0.05$. These analyses were carried out using SAS (SAS Institute Inc., Cary, North Carolina).

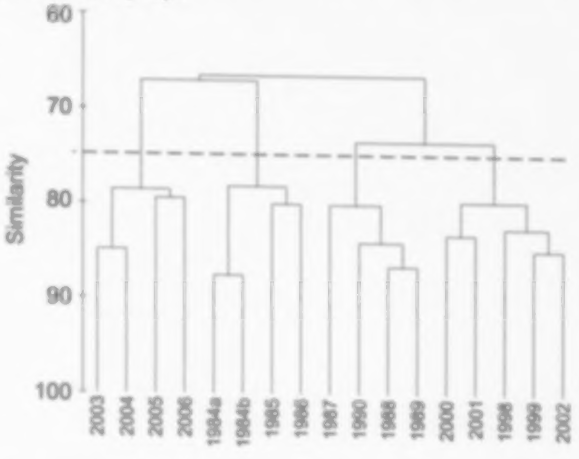


Fig. 2. Results of hierarchical cluster analysis, using group averages, on standardized, lake-wide, average crustacean biomass for 1984–2006. Zooplankton tows from two cruises were available in 1984. A similarity value of 0.75 (broken line) was used to delineate cluster groups.

Results

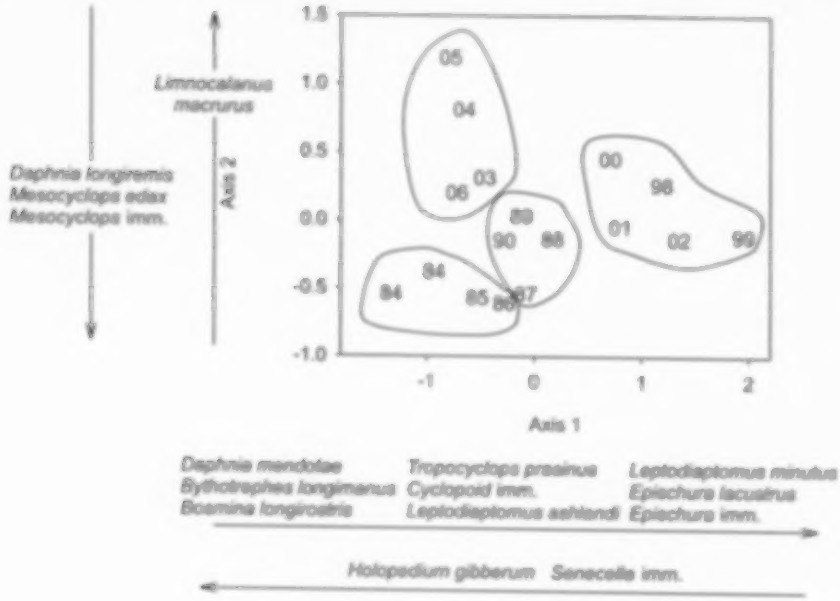
Crustacean community composition

Cluster analysis of historic zooplankton communities divided the time series into four periods, at a similarity value of 0.75: 1984–1986, 1987–1990, 1998–2002, and 2003–2006 (Fig. 2). These groupings were also apparent in the MDS ordination plot, in which no overlap was seen between the periods (Fig. 3). An ANOSIM procedure on the ordination results confirmed the significant differences between groups at $P < 0.001$. Both total crustacean biomass and total cladoceran biomass showed significant positive correlations with axis 1 scores, while neither total cyclopoid nor total calanoid biomass was correlated with either axis. Biomass of the two dominant cladocerans, *Daphnia mendotae* and *Bosmina longirostris*, and the predatory *Bythotrephes longimanus*, as well as the two most abundant diaptomids, *Leptodiaptomus ashlandi* and *Leptodiaptomus minutus*, also showed significant positive correlations with axis 1 scores, while the opposite trend was seen for the large-bodied non-daphnid cladoceran *Holopedium gibberum*. The two low-biomass periods 1984–1986 and 2003–2006 were separated in part by biomass of the large, deep-living calanoid *Limnocalanus macrurus*, which was positively correlated with axis 2 scores and thus higher in the later period. The loss of *Daphnia longiremis* and *Mesocyclops edax* contributed to the difference between the time periods 1984–1986 and 1987–1990, reflecting changes in species composition brought about by the appearance of *Bythotrephes longimanus* in the lake (Barbiero and Tuchman 2004).

Changes in crustacean biomass

Both basins exhibited similar trends in total crustacean biomass over time, although biomass in the southern basin was on average about 1.5 times higher than that in the northern basin (Figs. 4*a*, 4*b*). In both basins, total crustacean biomass was statistically significantly greater during 1998–

Fig. 3. Nonmetric multidimensional scaling of lake-wide, average summer crustacean zooplankton biomass from 1984 to 2006. Two cruises were conducted in 1984 and were averaged separately. Polygons indicate group membership resulting from hierarchical cluster analysis. Taxa significantly ($\alpha = 0.01$) correlated with each axis, using Spearman rank correlations, are shown along arrows indicating direction of increasing biomass.



2002 than in any other period; the only other significant difference found was lower biomass in 2003–2006 compared with 1988–1990 in the southern basin (Tables 1, 2). Between-period differences were much more pronounced in the case of cladoceran biomass (Figs. 4*c*, 4*d*). In 2003, there was a dramatic decline in cladoceran biomass to levels less than half of what had been seen in 1984–1990, and biomass remained low through 2006. In 2003 in the northern basin, and in 2005 in both basins, virtually no daphnids were found, a situation unprecedented in our data set. Biomass of both daphnid and non-daphnid, nonpredatory cladocerans was significantly lower in 2003–2006 than during any other period (Table 2). Overall, average nonpredatory cladoceran biomass in the northern and southern basins during the period 2003–2006 was 47% and 32.3%, respectively, of average levels during the period 1984–1990, and approximately 10% and 7.7%, respectively, of average levels during 1998–2002. No differences in predatory cladoceran biomass were found between any time periods (Tables 1, 2).

Unlike cladocerans, cyclopoid biomass was not consistently higher during the late 1990s – early 2000s compared with the 1980s (Figs. 4*e*, 4*f*). However, in 2005, cyclopoid copepod biomass exhibited a dramatic decline. As with the cladocerans, cyclopoid biomass in 2005 was lower than had been seen previously in our data set and remained depressed in 2006. Average cyclopoid biomass in those 2 years was 15.3% and 9.3% of levels seen in 1984–1990 and 10.4% and 6.5% of those seen in 1998–2002 for the northern and southern basins, respectively. As a result, cyclopoid biomass during 2003–2006 was significantly lower than that during any other period (Tables 1, 2). In contrast, total calanoid copepod biomass has remained relatively constant throughout our data set (Figs. 4*g*, 4*h*). No statistical differences in biomass between periods were found in the southern basin (Table 1), while in the northern basin, biomass in 1987–1990 and 2003–2006 was higher than that in 1984–1986, but lower than that in 1998–2002 (Tables 1, 2).

Shifts in size distribution

The shifts in community composition were accompanied by dramatic shifts in the size distribution of crustacean biomass when comparing 1998–2002 with 2003–2006 (Fig. 5). During the earlier period, nonpredatory cladocerans contributed a substantial proportion (42% and 65% for the northern and southern basins, respectively) of the total crustacean biomass contained in individuals greater than 0.9 mm. For the years 2003–2006, this percentage dropped to 10% and 18% for the northern and southern basins, respectively, of which nearly half was accounted for by *Holopedium gibberum*. The reductions in crustacean biomass were most notable in the 1.1–2.2 mm size range. In both basins, average biomass during 2003–2006 was reduced by over 80% in this size range compared with 1998–2002.

With the reductions in cladocerans, and in particular of *Daphnia*, a greater percentage of large (>0.9 mm) crustacean biomass was contributed by *Limnocalanus macrurus* from 2003 onwards. However, while the relative contribution of this species to large crustacean biomass increased notably, from 18% and 9% in 1998–2002 to 42% and 30% in 2003–2006 for the northern and southern basins, respectively, the actual biomass of this species exhibited only modest increases between the two periods.

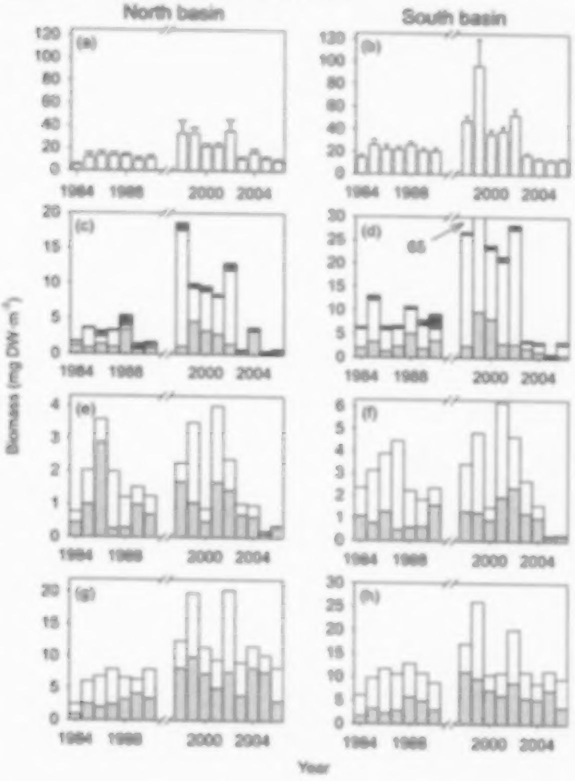


Fig. 4. Mean biomass (mg dry weight (DW)·m^{-3}) of (*a*, *b*) total crustaceans, (*c*, *d*) cladocerans, (*e*, *f*) cyclopoid copepods, and (*g*, *h*) calanoid copepods during August cruises in the northern (right panels) and southern (left panels) basins of Lake Huron, 1984–2006. For panels (*c*) and (*d*), light gray indicates non-daphnid, nonpredatory cladocerans (largely *Bosmina* and *Holopedium*), open bars indicates species of *Daphnia*, dark gray indicates predatory cladocerans; for panels (*e*), (*f*), (*g*), and (*h*), shading indicates adults, and open bars indicates immatures (copepodites). Note differences in scale between the two basins.

Decline in *Diporeia*

The declines in non-calanoid crustacean biomass roughly corresponded to declines in the amphipod *Diporeia* at stations of comparable depths in the two basins (Fig. 6). Particularly notable declines were seen between 2002 and 2004, years bracketing the shifts in the zooplankton community. During this period, estimated areal densities of offshore *Diporeia* populations decreased by 83% and 80% in the northern and southern basins, respectively. By 2006, *Diporeia* densities in the northern and southern basins were 15% and 3%, respectively, of 1997 values, the year in which sampling began.

Environmental variables

A number of environmental variables have shown long-term trends over the course of our time series consistent with decreasing trophic state (Fig. 7). Both spring and summer TP have declined significantly between 1984 and 2006 (Figs. 7*a*, 7*b*; Table 3), while Secchi depth has increased in both seasons (Figs. 7*c*, 7*d*; Table 3). Soluble silica has also increased during both seasons, with an apparent step change occurring in 2003 (Figs. 7*e*, 7*f*; Table 3). Chlorophyll *a*, however, has shown no consistent trends (Figs. 7*g*, 7*h*; Table 3). When just the two most recent periods 1998–2002 and 2003–2006 are considered, the same trends are seen during the spring for all variables (Table 4). However, statistically significant differences were not seen between the periods 1998–2002 and 2003–2006 for TP in the northern basin or for Secchi depth in either basin during the summer (Table 4).

Table 1. Results of generalized linear model analyses on total crustacean biomass, non-daphnid cladoceran biomass (excluding predatory cladocerans), daphnid cladoceran biomass, predatory cladoceran biomass, cyclopoid copepod biomass, and calanoid copepod biomass.

Variable	Basin × period	Basin	Period
Total crustacean	$P = 0.006$	N	$P = 0.001$
		S	$P < 0.001$
Non-daphnid	NS	All	$P = 0.007$
Daphnid	$P < 0.001$	N	$P < 0.001$
		S	$P < 0.001$
Predatory cladoceran	NS	All	NS
Cyclopoid	NS	All	$P = 0.016$
Calanoid	$P = 0.002$	N	$P = 0.003$
		S	NS

Note: Basin × period indicates the probability level of that interaction, if significant ($\alpha = 0.05$; NS, not significant.). Where a significant interaction was found, the two basins were analyzed separately, and results were reported for each basin (indicated by N for northern basin and S for southern basin); otherwise a single analysis was conducted (indicated by All). Period indicates probabilities of significant differences between time periods.

Surface water temperature has shown considerable variation over the course of our time series (Figs. 7*i*, 7*j*), and in both basins and during both seasons, temperatures during 1998–2002 were significantly higher than those during 2003–2006 (Table 4), although during the summer these differences were slight. Since uptake of silica by diatoms is highly seasonal, and thus potentially impacted by small differences in seasonal timing of surveys, the effects of interannual differences in temperature for the period 1998–2006 were removed by regressing SiO_2 values against temperature and examining the residuals (Fig. 8). Even after taking into account temperature in this way, a very distinct step change was apparent in 2003 in both the spring and the summer.

Discussion

The crustacean zooplankton community in Lake Huron exhibited a sudden shift in both biomass and composition in 2003 when the two dominant cladocerans, *Daphnia mendotae* and *Bosmina longirostris*, virtually disappeared. These declines and the subsequent decline in cyclopoid copepod biomass are without precedent in our data set.

Evidence for planktivory

At first glance, our data bear some hallmarks of planktivory. Most planktivores are size-selective, preferentially consuming the largest available individuals and thereby shifting the size structure of zooplankton communities to-

Table 2. Results of pairwise Tukey–Kramer comparisons between time periods for total crustacean biomass, non-daphnid cladoceran biomass (excluding predatory cladocerans), daphnid cladoceran biomass, cyclopoid copepod biomass, and calanoid copepod biomass.

Variable	Pairwise comparison results			
Total crustacean (N)	1998–2002	1987–1990	2003–2006	1984–1986
Total crustacean (S)	1998–2002	1987–1990	1984–1986	2003–2006
Non-daphnid (N, S)	1998–2002	1987–1990	1984–1986	2003–2006
Daphnid (N, S)	1998–2002	1984–1986	1987–1990	2003–2006
Cyclopoid (N, S)	1998–2002	1984–1986	1987–1990	2003–2006
Calanoid (N)	1998–2002	2003–2006	1987–1990	1984–1986

Note: For each variable, time periods are arranged from highest to lowest biomass. Basins that results apply to are indicated in parentheses (N, northern basin; S, southern basin). Time periods that are not significantly different at α = 0.05 are linked by underlining.

Fig. 5. Size distribution of non-daphnid cladoceran (hatched bars), *Daphnia* (open bars), cyclopoid copepod (gray bars), and calanoid copepod (black bars) biomass for the northern (left panels) and southern (right panels) basins of Lake Huron for August cruises, 1998–2006. Note that *Limnocalanus macrurus* accounts for all calanoid biomass > 2.4 mm.

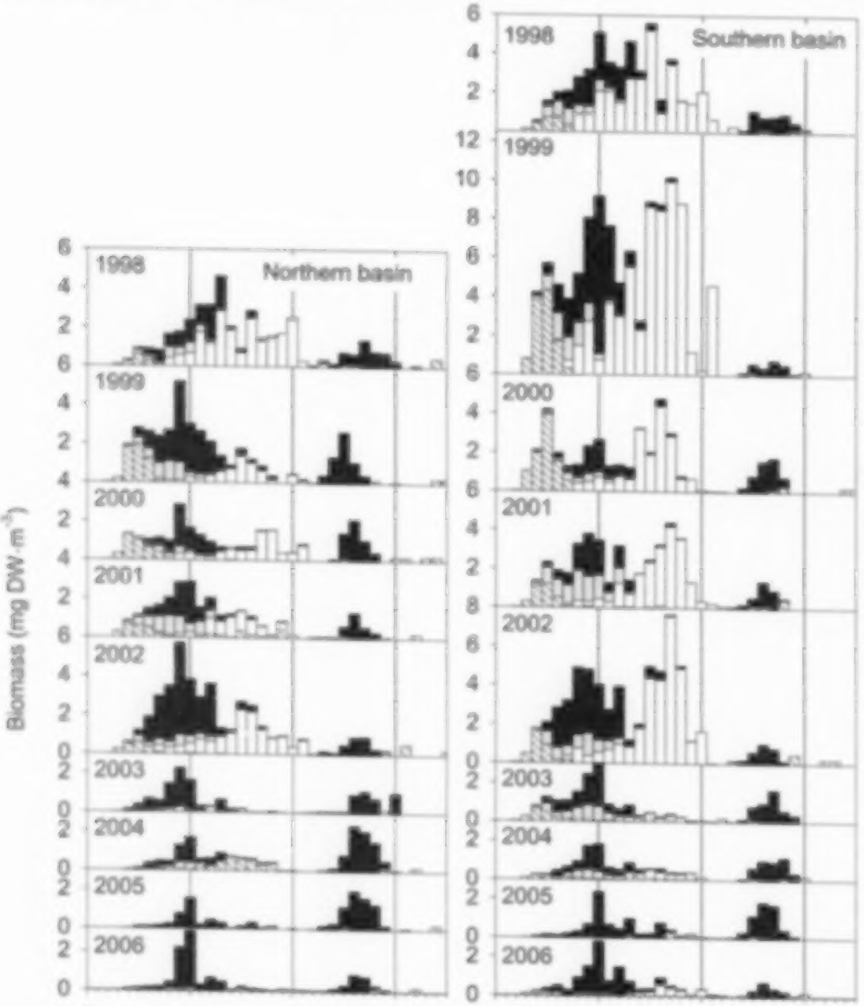
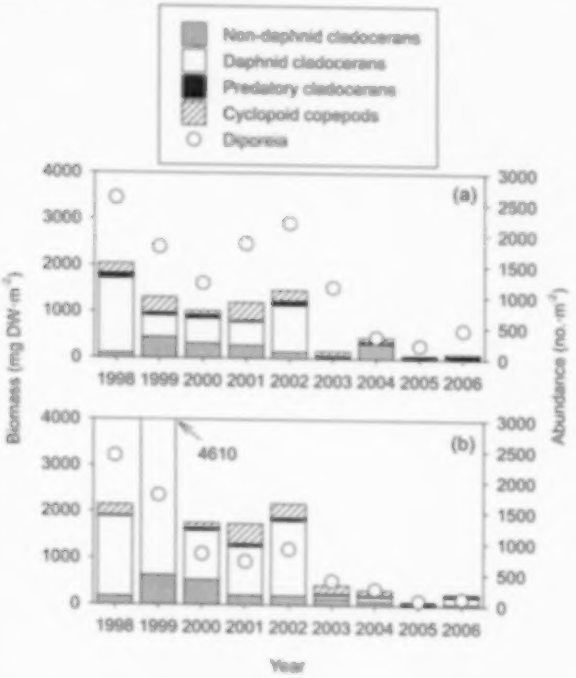


Fig. 6. Stacked bar plot (left axis) of areal August non-daphnid cladoceran biomass (excluding predatory cladocerans), daphnid cladoceran biomass, predatory cladoceran biomass, and cyclopoid copepod biomass (adults and copepodites); scatterplot (right axis) of *Diporeia* areal densities for the (*a*) northern and (*b*) southern basins of Lake Huron, 1998–2006.



wards smaller individuals (Brooks and Dodson 1965; Galbraith 1967; Hutchinson 1971). However, susceptibility of zooplankton to predation is also dependent upon capture probabilities, which vary as a function of the relative swimming strength and the characteristic movements of different zooplankton taxa (Drenner et al. 1978). In general, cladocerans are most easily captured, followed by cyclopoid copepods and then calanoid copepods (Drenner and McComas 1980; Zaret 1980; Link 1996). Therefore, increasing predation pressure might be expected to sequentially remove first larger cladocerans, then smaller cladocerans, and ultimately cyclopoid and calanoid copepods. The disappearance of both large and small cladocerans in 2003 in the northern basin and in 2004 in the southern basin of Lake Huron, therefore, would suggest intense planktivory, as would the subsequent disappearance of cyclopoid copepods in both basins.

There is evidence that planktivory by prey fish can substantially alter zooplankton community structure in the Great Lakes. In the mid-1960s, large populations of alewives in Lake Michigan coincided with reductions in large cladocerans, such as *Leptodora kindtii*, *Daphnia galeata*, and *Daphnia retrocurva*, as well as some large calanoids, and increased the densities of some smaller species of zooplankton, such as *Daphnia longiremis* and *Bosmina longirostris* (Wells 1970). After a subsequent alewife decline, the zooplankton community shifted back towards its composition before alewives. A similar, though somewhat more subtle, series of shifts in offshore zooplankton communities was seen in the early 1980s, when a relaxation in alewife predation pressure is thought to have both allowed the establishment of the large *Daphnia pulicaria* and to have increased body lengths of *Daphnia retrocurva* and *Daphnia galeata mendotae* (Evans and Jude 1986).

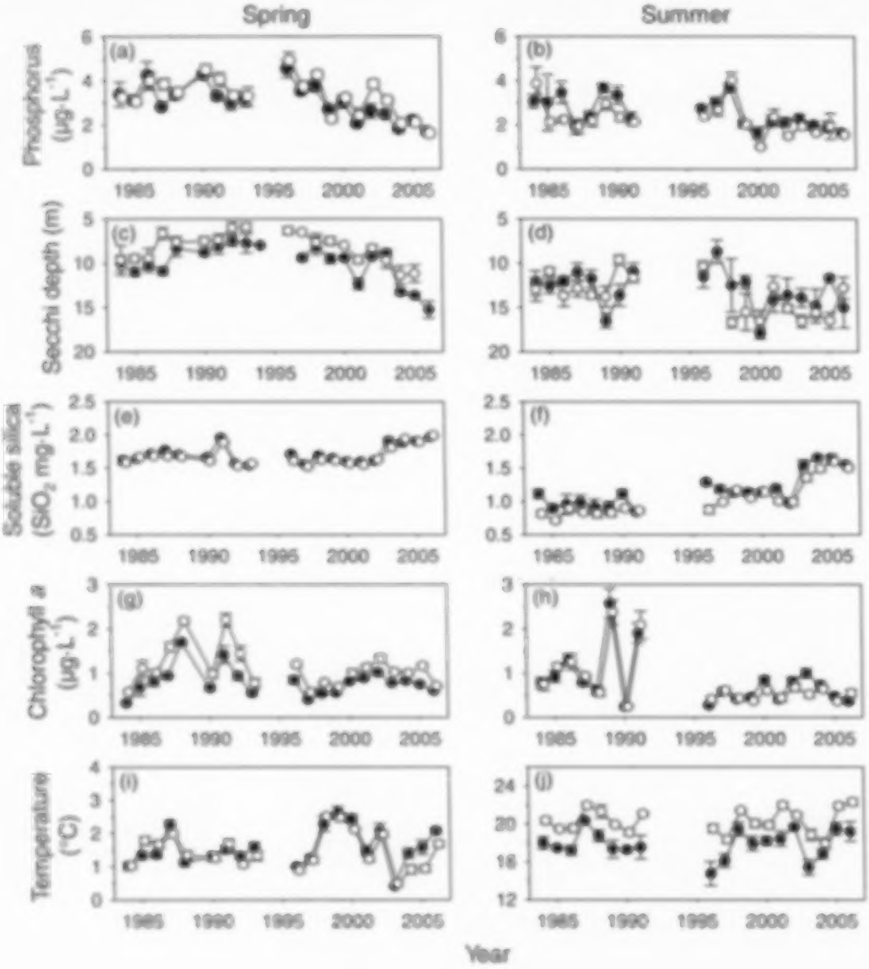
The shifts we document here have been far more dramatic, involving not merely a shift in cladoceran size distribution, but a near total elimination of cladoceran biomass in all size categories in some years, as well as substantial declines in cyclopoid copepods. It would seem to suggest a very marked and sustained increase in planktivory starting in 2003. However, alewife populations are currently at historic lows following the collapse of the adult population in 2003, and overall prey fish biomass remains low (Roseman et al. 2008). The declines in zooplankton reported here are thus ostensibly difficult to reconcile with the current prey fish community.

However, in spite of the current low population densities of adult prey fish, we believe that planktivory could still be at least partially responsible for the declines in zooplankton densities through predation by young-of-the-year planktivores. The 2003 year class of alewife was the largest seen in the US Geological Survey (USGS) data series, which goes back to 1992 (Roseman et al. 2008). Young alewives prey exclusively on zooplankton (Morsell and Norden 1968; Hewett and Stewart 1989) and would have begun exploiting larger cladocerans such as *Daphnia* by the time of our August survey in 2003, so indications of intense planktivory might be expected in that year. Similarly, record numbers of both juvenile bloaters and rainbow smelt were seen in the lake in 2005 (Roseman et al. 2008), a year that also saw the lowest cladoceran biomass in our data series. Like alewives, juvenile bloater (Crowder and Crawford 1984; Warren and Lehman 1988), and to a lesser extent rainbow smelt (Johnson et al. 1998; Dermott et al. 1999), consume mostly zooplankton, so they would have a disproportionate impact on the zooplankton community compared with older fish. The impact of age-0 fish might be even greater than is apparent from the USGS survey data, since these fish tend to remain higher in the water column (Wells 1968*a*) and thus are underestimated in bottom trawl data.

Potential impact of declines in *Diporeia*

The unusually large year classes of alewife in 2003 and of bloater and rainbow smelt in 2005 might help to explain the low cladoceran biomass seen in those 2 years, but it is still paradoxical that such pronounced decreases in edible zooplankton should have persisted in 2004 and 2006, years in which neither juvenile nor adult prey fish biomass was particularly high. Between 2002 and 2004, however, populations of *Diporeia* in the offshore of Lake Huron exhibited particularly notable declines. While the initial declines in zooplankton in 2003 might have been due to the large year class of alewife, we believe that decreases in *Diporeia* have contributed to keeping subsequent zooplankton populations low by shifting diets of older prey fish. *Diporeia* make up a substantial portion of the diets of all three major prey fish found in Lake Huron. Young alewife in Lake Michigan consume mostly zooplankton, while *Diporeia*

Fig. 7. Spring (left panels) and summer (right panels) (*a*, *b*) total phosphorus, (*c*, *d*) Secchi depth, (*e*, *f*) soluble silica, (*g*, *h*) chlorophyll *a*, and (*i*, *j*) surface water temperature for the northern (solid symbols) and southern (open symbols) basins of Lake Huron. Error bars indicate one standard error.



makes up an increasingly larger portion of the diet of larger fish (Wells 1980; Hondorp et al. 2005), particularly in spring when *Diporeia* are higher in the water column (Wells 1968*b*) and thus more easily captured by the mainly planktivorous alewife (Pothoven and Vanderploeg 2004; Hondorp et al. 2005). Morsell and Norden (1968) showed that *Diporeia* made up 80% of the bulk of total food consumed by alewife in Lake Michigan during 1966–1967. Similarly, after an initial period during which the diet is dominated by zooplankton, bloaters increasingly exploit the benthos, in particular *Diporeia* and *Mysis*. Rand et al. (1995) found that *Diporeia* comprised 80% of the spring diet of age-2 and older bloater in Lake Michigan, while *Diporeia* and *Mysis* were found to contribute the vast majority of the diet of bloaters greater than 8 mm in a study by Wells and Beeton (1963). In Lake Superior, *Diporeia* made up 82% by weight of the diet of adult smelt in the Apostle Islands region of Lake Superior (Selgeby et al. 1994).

The declining offshore *Diporeia* population, particularly after 2002, could have contributed not only to the decline in adult alewife in Lake Huron, but also to the decline in zooplankton biomass by forcing the remaining prey fish to utilize alternate prey items. This could be quite important given not only the large difference in size between *Diporeia* and zooplankton (approximately 333:1 dry weight; Morsell and Norden 1968), but also the difference in energy content (4429 and 1674 $J{\cdot}g^{-1}$ for *Diporeia* and cladocerans, respectively; Stewart and Binkowski 1986). According to these estimates, it would take approximately 882 zooplankton to provide the energy equivalent of one *Diporeia*. Alewives are known to be flexible feeders that will utilize alternate food items when preferred prey are unavailable. For example, in *Diporeia*-depleted zones of Lake Michigan, large alewife did not exhibit reductions in stomach food content, but instead increased their consumption of zooplankton (Hondorp et al. 2005). Additionally, competition with alewives results

Table 3. Results of Mann–Kendall nonparametric trends analyses on total phosphorus (TP), Secchi depth (Secchi), soluble silica (SiO_2), and chlorophyll *a* (Chl) in the northern (N) and southern (S) basin of Lake Huron.

Cruise	Variable	Basin	*Z*	*P*	Trend
Spring	TP	N	–6.152	<0.0001	–
		S	–5.863	<0.0001	–
	Secchi	N	2.076	0.0380	+
		S	4.296	<0.0001	+
	SiO_2	N	2.075	0.0380	+
		S	2.396	0.0166	+
	Chl	N	–1.691	NS	
		S	–3.623	0.0003	–
Summer	TP	N	–2.755	0.0059	–
		S	–3.639	0.0003	–
	Secchi	N	2.153	0.0310	+
		S	2.832	0.0046	+
	SiO_2	N	5.910	<0.0001	+
		S	9.167	<0.0001	+
	Chl	N	–1.157	NS	
		S	–0.464	NS	

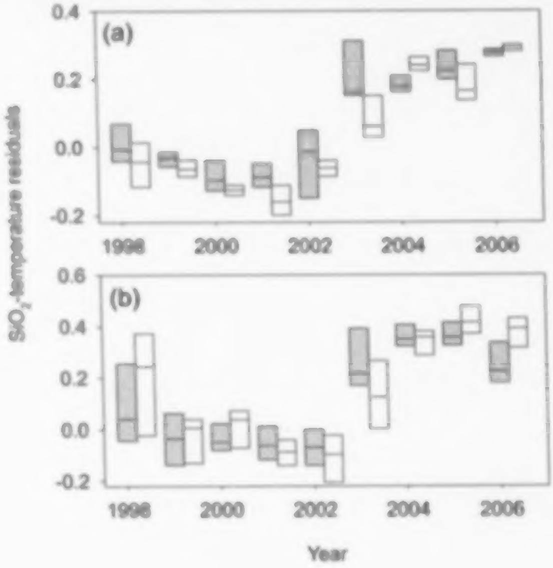
Note: *Z* values are given, along with associated probability (*P*) and direction of trend (+, increasing; –, decreasing) where significant ($\alpha = 0.05$) trends were found.

Table 4. Analysis of variance results between the time periods 1998–2002 and 2003–2006 for total phosphorus (TP), Secchi depth (Secchi), soluble silica (SiO_2), chlorophyll *a* (Chl), and surface water temperature (Temp).

Cruise	Variable	Basin	*F*	*P*	Period
Spring	TP	N	88.5	0.0002	1998–2002
		S	55	0.0002	1998–2002
	Secchi	N	224.9	0.0006	2003–2006
		S	25.8	0.0070	2003–2006
	SiO_2	N	307	<0.0001	2003–2006
		S	594	<0.0001	2003–2006
	Chl	N	0.0002	NS	—
		S	5.09	NS	—
	Temp	N	114	0.0001	1998–2002
		S	376	<0.0001	1998–2002
Summer	TP	N	2.79	NS	—
		S	8.63	0.0220	1998–2002
	Secchi	N	0.008	NS	—
		S	0.75	NS	—
	SiO_2	N	142	<0.0001	2003–2006
		S	95.3	<0.0001	2003–2006
	Chl	N	7.96	0.0370	2003–2006
		S	0.13	NS	—
	Temp	N	9.3	0.0280	1998–2002
		S	6.8	0.0350	1998–2002

Note: In cases were a significant difference was found, the period with the higher mean value for each variable is indicated in the column Period. In all cases, $\alpha = 0.05$.

Fig. 8. Box plots of residuals of least squares regression analysis between surface water temperature and soluble silica (as SiO_2) for (*a*) spring and (*b*) summer in the northern (shaded boxes) and southern (open boxes) basins of Lake Huron. Boxes indicate 25th and 75th percentiles; center lines indicate medians.



in bloaters shifting to a more benthic habit earlier in life (Crowder and Crawford 1984), so the reductions in adult alewife densities in Lake Huron could result in greater planktivory by bloaters. However, it is unclear whether there is currently enough consumptive demand amongst prey fish, taking into account both age-0 fish and declines in *Diporeia*, to explain the greatly reduced crustacean biomass in the lake.

Invertebrate predation

While the main invertebrate predator in the lake, *Bythotrephes longimanus*, can have substantial impacts on crustacean community size and composition (Yan et al. 2001; Boudreau and Yan 2003), it is unlikely to have contributed to the zooplankton community changes after 2002 seen in our study, since populations were not significantly different between 2003–2006 and the preceding 5 years. Additionally, *Daphnia mendotae*, which nearly disappeared in Lake Huron since 2003, has consistently been shown to persist in the face of *Bythotrephes* invasions in North America (Yan et al. 2001; Barbiero and Tuchman 2004; Strecker et al. 2006).

Lake Huron also supports populations of the opossum shrimp, *Mysis relicta* (Teter 1960), which is strongly carnivorous in the lake at later life stages (Branstrator et al. 2000). Mysids have been implicated in the control of cladoceran populations in Lake Washington (Edmondson and Litt 1982) and Lake Tahoe (Goldman et al. 1979). Unfortunately, reliable times series data do not exist on *Mysis relicta*, so its role in the zooplankton community shifts is unknown.

Trophic changes – *Dreissena* impacts

An alternative explanation for the observed shifts in zooplankton is that a reduction in food supply, through either continued reductions in nutrient loadings to the lake and (or) filtration–benthification processes by expanding *Dreissena bugensis* populations, has exerted bottom-up controls on crustacean community size. Our data provide some evidence for this. The trophic state of Lake Huron has changed conspicuously during the course of this study, with TP concentrations in the open waters of the lake declining by approximately 60% between 1996 and 2004, although most of this decline occurred prior to 2000. Spring Secchi depth has increased in both basins in recent years, roughly corresponding to the decrease in phosphorus, and in the past 3 years, transparency in the northern basin has been unusually high.

Soluble silica has also increased, which is suggestive of a reduction in diatom productivity. Unlike the other trophic state variables, however, the soluble silica increases in both spring and summer were apparent as a step change occurring in 2003, the same year that saw the major declines in cladoceran biomass. If in fact this increase in soluble silica were due to a sharp decrease in diatom production starting in 2003, this would lend support to a declining food supply as a contributory factor in the zooplankton demise, particularly since diatoms dominate the Lake Huron phytoplankton community in April and can contribute approximately half of phytoplankton biovolume in August (Barbiero and Tuchman 2001; Barbiero and Tuchman 2002). Increases in soluble silica are an expected consequence of long-term oligotrophication in large lakes, as increases in phosphorus limitation reduce spring diatom production and thus sedimentation and long-term loss of particulate silica, a phenomenon recently documented in Lake Michigan (Barbiero et al. 2002). The marked increase in silica starting in 2003 could also be indicative of increased grazing pressure (e.g., by dreissenids). Similar increases in soluble silica, coinciding with increases in dreissenid populations, have been seen in the eastern basin of Lake Erie (Barbiero et al. 2006), which we have interpreted as being a consequence of limitation of phytoplankton by dreissenid grazing.

Populations of *Dreissena bugensis* have indeed expanded substantially in Lake Huron between 2000 and 2003 at intermediate (31–50 m) depths (Nalepa et al. 2007). Densities at depths > 50 m have further increased nearly fivefold between 2003 and 2007 (T. Nalepa, Great Lakes Environmental Research Lab, NOAA, 2205 Commonwealth Boulevard, Ann Arbor, MI 48105, USA, personal communication, 2008); however, mussels are still extremely rare at the depths (45–130 m) corresponding to our sampling locations, and enumeration of dreissenids from GLNPO benthos samples collected between 2003 and 2006 indicate a near total absence of mussels at our benthos sites (D.C. Rockwell, unpublished data). Mussels might still impact the offshore through both sequestration of nearshore nutrients from nonpoint sources and nutrient stripping from offshore waters periodically advected through the nearshore zone (Hecky et al. 2004).

Even if not the sole cause of the zooplankton decline, decreasing primary production — or its diversion to the benthos — could still have served to heighten impacts of increased predation pressure on zooplankton. A reverse example of this was seen in Lake Tahoe, where predation by the newly introduced *Mysis relicta* resulted in the disappearance of all cladocera in 1970 (Richards et al. 1975). By 1983, however, steadily increasing productivity had enabled *Daphnia rosea* to co-exist with *Mysis relicta* in the lake (Byron et al. 1986). The influence of overall productivity in mediating impacts of planktivory in Lake Huron is perhaps apparent in the differences seen between the two basins; greater shifts in the zooplankton community were consistently seen in the less productive northern basin compared with the more productive southern basin.

Multiple causes probably responsible for zooplankton declines

Given the data currently available, no single mechanism seems sufficient to explain the shifts in the zooplankton community observed in Lake Huron in 2003. The convergence of a number of factors has likely resulted in the observed overall decline in zooplankton biomass in Lake Huron. First, record year classes of prey fish in 2003 and 2005 have could have increased predation pressure in those years, because of the obligate planktivory of these young fish. Second, the declines in *Diporeia* after 2002 have likely intensified the planktivory of the remaining adult prey fish, in spite of the lower overall biomass of these fish. Finally, compounding these factors, the declining trophic state of the lake has likely reduced secondary production, resulting in a smaller base of crustacean biomass to support this intensified planktivory. Dreissenid mussels may be implicated in several of these factors (e.g., through nutrient sequestration and antagonistic interactions with *Diporeia*).

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The importance of scale for fishing impact estimations

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Abstract: The impact of a bottom trawl fishery on fish or benthos is often determined by multiplying the frequency of the passing of the trawl by a factor for the effect (i.e., % mortality) of the singular passing of the gear. As fishing intensity in an area is not homogeneously distributed, it is necessary to determine the proportions of the area that are fished with different trawling frequencies, as these subareas together contribute to the overall species' mortality. In this study, we show that the perceived proportion of the area fished with a specific trawling frequency depends upon the spatial and temporal scale used. A smaller spatial scale results in an increased perceived patchiness of the fishing intensity, while a longer time period does the opposite. The implication is that to determine the fishing-induced mortality of a particular species, the trawling frequency needs to be determined at those spatio-temporal scales that are appropriate considering the species' spatial processes (e.g., dispersion) or temporal processes described by life history characteristics.

Résumé : L'impact de la pêche au chalut de fond sur les poissons ou sur le benthos se détermine souvent en multipliant la fréquence de passage du chalut par un facteur représentant l'effet (par ex., % de mortalité) d'un passage unique de l'engin. Comme l'intensité de pêche dans une région n'est par répartie de manière homogène, il est nécessaire de déterminer les proportions de la surface qui ont été soumises aux différentes fréquences de chalutage, puisque ces sous-surfaces contribuent ensemble à la mortalité globale de l'espèce. Dans notre étude, nous montrons que la perception de la proportion de surface soumise à une intensité spécifique de chalutage dépend de l'échelle spatiale et temporelle utilisée. Une échelle spatiale réduite a pour résultat la perception d'une contagion accrue de l'intensité de pêche, alors qu'une période de temps plus longue a l'effet contraire. Les conséquences de cette observation est que, pour déterminer la mortalité reliée à la pêche chez une espèce en particulier, il est nécessaire de déterminer la fréquence de chalutage aux échelles spatiotemporelles appropriées, compte tenu des processus spatiaux (par ex. la dispersion) et temporels de l'espèce décrits par les caractéristiques de son cycle biologique.

[Traduit par la Rédaction]

Introduction

With the development of an ecosystem approach to fisheries management, research has increasingly focused on the environmental effects of fishing on nontarget fauna and marine habitats (Hall 1999; Sinclair and Valdimarsson 2003) as well as on management scenarios or options (e.g., effort reduction, closed areas, technical measures) that take these impacts into account (Brodziak and Link 2002; Link et al. 2002; Jennings 2005). Therefore, more than in traditional fisheries management, this necessitates a reappraisal of the issue of scale in space and time when estimating fishing impact.

Many studies that estimated fishing impact emphasized the importance of the spatial component even though the spatial scale at which they studied the relationship differed (e.g., Rijnsdorp et al. 1998; Ragnarsson and Steingrimsson 2003; Bellman et al. 2005). Rijnsdorp et al. (1998) found that beam trawling was patchily distributed up to a spatial resolution of approximately 1 nautical mile (n.mi.) × 1 n.mi. (1 n.mi. = 1.852 km), but randomly distributed at higher resolutions, and discussed the relevance of this when studying the impact on benthic organisms. Therefore several studies used 1 n.mi. × 1 n.mi. or similar resolutions when estimating the impact of trawling on the benthic fauna (e.g., Piet et al. 2000; Dinmore et al. 2003; Hiddink et al. 2006*a*, 2006*b*), while others (Marchal et al. 2006) used a scale of International Council for the Exploration of the Seas (ICES) rectangles (area is approximately 30 n.mi. × 30 n.mi.) to describe the relationship between fishing mortality of commercial fish species and effort. Dinmore et al. (2003) studied the microscale distribution of fishing at scales of 1 × 1, 2 × 2, 4 × 4, and 8 × 8 n.mi. and observed that the spatial scale may have a critical effect on any interpretation of fishing impacts but did not further analyze this. Piet et al. (2007) showed that estimates of fishing mortality based on a 1 × 1 n.mi. scale differed from those based on ICES rectangles (approximately 30 n.mi. × 30 n.mi.) but did not examine or attempt to describe the mechanism. Mills et al. (2007) showed that trawling effort could be reported as area impacted per unit time per unit area at a range of grid scales from 1 km to 100 km and decided that for vessel monitoring through satellite (VMS) data with a 2 h interval, trawling effort is accurately represented at a grid cell resolution of 3 km or less. All these studies determined fishing impact based on yearly estimates of trawling intensity without con-

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Table 1. Codes used for the spatial scale.

Code	Description	No. of units	Surface area (km^2)
1	ICES rectangle	1	3086.914
3	3×3	9	342.990
10	10×10	100	30.869
30	30×30	900	3.430
100	100×100	10 000	0.309
300	300×300	90 000	0.034
1000	1000×1000	1 000 000	0.003

Note: The International Council for the Exploration of the Seas (ICES) rectangle is divided into an increasingly large number of units whose individual surface area was calculated by assuming the rectangle was 30 nautical miles (n.mi.) × 30 n.mi. (= 3086.914 km^2).

Table 2. Codes used for the temporal scale.

Code	Description	No. of subunits
qtr	4 quarters per year, period 1994–2005	48
1	1994, 1995, 1996, 1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005	12
2	1994–1995, 1996–1997, 1998–1999, 2000–2001, 2002–2003, 2004–2005	6
5	1994–1998, 1999–1903	2
10	1994–2003	1

sidering different time scales. Eastwood et al. (2007) recognized this issue but indicated that they made no attempt to introduce a temporal component to their pressure assessment because of the complications of developing a common metric. Thus, there are several studies that have explored the relationship between effort and impact, often taking into account the spatial distribution of the fishery. However, none of them have fully addressed the issue of spatial or temporal scale, let alone the combination.

Spatial and temporal scales at which the impact on the system is determined must match those of ecological patterns and processes (Marceau 1999). The above studies indicate that the choice of the appropriate scale depends on the ecosystem component, where studies on fish often use larger scales (Marchal et al. 2006), while studies on benthos use smaller scales (km^2 or n.mi.^{2}, e.g., Dinmore et al. 2003; Hiddink et al. 2006*a*, 2006*b*). For habitats like coral reefs, a resolution of square metres may be most appropriate (Andrews and Anderson 2004; Fox and Caldwell 2006).

The aim of this paper is to demonstrate the importance of the spatio-temporal scale when assessing the impact of a bottom trawl fishery by showing that the proportion of an area that is trawled with a certain frequency is not only determined by the total amount of effort exerted on that area but also by the spatio-temporal scale chosen. We therefore will argue that the models used to assess fishing impact on a specific ecosystem component should use appropriate scales in both space and time. The choice of the appropriate scale may depend on spatial processes such as migration or dispersion or temporal processes described by life history characteristics that are specific for that ecosystem component.

Material and methods

For this study, we used what is probably the longest time series of high-resolution spatial distribution data of fishing activity in European waters: the combined automatic position registration (APR) and VMS database of the Dutch beam trawl fleet in the southeastern North Sea. This data set commences in 1993 and continues until present and has been previously described by Rijnsdorp et al. (1998) and Piet et al. (2000, 2007). We combined this data set with the VIRIS database, which contains information on fishing activities of the entire Dutch fleet at a spatial resolution of ICES rectangles stored in individual fishers' EC logbooks. The data set is described in more detail in Piet et al. (2007). This study is based on the period 1994–2005.

To study the effects of the spatial and temporal scale, we aggregated the fishing registrations into spatial and temporal units at different scales. For the spatial scale, we used the ICES rectangle as the basic spatial unit, as this is also the scale at which effort data are collected and divided into increasingly smaller subunits (Table 1). For the temporal scale, we aggregated the registrations into units of quarterly, 1-year, 2-year, 5-year, and 10-year periods (Table 2).

The similarity in spatial distribution over time of the entire Dutch fleet was assessed at different spatial scales. To do this, we cumulated all registrations in a year for each spatial unit corresponding to a selected spatial scale. Then, the Pearson's correlation coefficient was calculated for the number of registrations by spatial unit in one year compared with another year, using different time intervals between years (1 = two consecutive years, e.g., 1995 vs. 1996 or 2003 vs. 2004; 10 = 1994 vs. 2004 or 1995 vs. 2005). Similarity was expressed as the mean Pearson's correlation coefficient of all pairwise year comparisons in each of the 1- to 11-year time intervals or as the unique coefficient for the 12-year time interval.

The fishing impact on an ICES rectangle was described by the total amount of effort and how this is distributed in terms of the proportion of the surface area of the rectangle that was trawled with a specific frequency (year^{-1}). The trawling frequency per spatio-temporal unit was calculated from the registrations in the APR–VMS database by calculating the equivalent swept area from the amount of effort (i.e., hours trawled) these registrations represent, the trawling speed, and width of the gear according to Piet et al. (2007) and dividing this by the area of the spatial unit (see Table 1). The following classes of trawling frequency were distinguished: 0, >0–0.1, >0.1–1, >1–10, >10–100, >100–1000, and >1000 times the unit surface area by year. The effect of spatial scale was assessed using a constant temporal scale of 1 year (code 1 in Table 2), while the effect of temporal scale was assessed for a spatial scale of 3.43 km^2 (code 30 in Table 1). As the observed effect of scale differed depending on the amount of effort exerted on the area (based on the VIRIS data), we distinguished three classes of fishing effort per ICES rectangle: low fishing effort (average 0–10 days at sea per year), medium fishing effort (average 50–100 days at sea per year), and high fishing effort (average 500–1000 days at sea per year).

Fishing impact on a population is often expressed as the annual mortality (%) caused by fishing (Piet et al. 2007). This is calculated by multiplying the frequency of the passing of the trawl by a factor for the effect (i.e., % mortality) of the singular passing of the gear, thereby recognizing that the first fishing event has proportionally more impact than subsequent ones because these only kill a proportion of the fauna that survived the previous event. The annual mortality (%) is consequently the sum of all consecutive impacts. For the effect of the singular passing of the gear on the population, we distinguished two virtual species that were evenly distributed within the ICES rectangle: one less vulnerable species with a 25% mortality and the other a more vulnerable species with a 75% mortality. We followed the same approach as when we determined the fishing impact on an ICES rectangle in terms of proportions trawled, but now we only distinguished two effort classes (i.e., low and high fishing effort).

Results

While the database based on individual fishers' EC logbooks uses a spatial scale of ICES rectangles to express fishing intensity, the data that are currently being collected using VMS allow a much higher spatial resolution. The fishing intensity of the Dutch beam trawl fleet in the southeastern North Sea shows distinct spatial patterns that determine how this fishery impacts the ecosystem. However, based on these data, our perception may change considerably depending on the spatial scale used (Fig. 1).

The spatially disaggregated information of fishing intensity is usually reported per year, which may be appropriate at the crude spatial scale of ICES rectangles. However, when the spatial resolution is increased (Fig. 1), the temporal scale at which the data are aggregated may become even more relevant when assessing the impact of a fishery.

An increase of temporal scale usually results in a more evenly distributed pattern of fishing activity. This is caused by the fact that the spatial distribution of fishing activity gradually shifts from one year to the next. An analysis of the similarity in spatial distribution at different scales between years at different intervals shows how the similarity decreases at increasingly larger intervals, and this decrease is stronger at higher spatial scales (Fig. 2). At the spatial scale of an ICES rectangle, there is a minimum similarity after some 10 years expressed by a correlation coefficient of 0.75; at a spatial scale of 3 × 3 spatial units per rectangle, this is about 0.4, while at higher scales this is only about 0.2–0.3.

The fishing impact on an ICES rectangle expressed as the proportions of the surface area that are trawled with a specific frequency (year^{-1}) is not only affected by the overall amount of fishing effort (expressed as number of days at sea) but also by the spatial and temporal scale chosen (Fig. 3). In an average rectangle that was fished with an effort of between 50 and 100 days at sea per year, the most commonly used high-resolution spatial scale of approximately 1 n.mi.^{2} or 3.43 km^2 (30 × 30 spatial units per rectangle) and a temporal scale of year would show that 34% of the rectangle was not fished, 8% was fished less than 0.1 year^{-1}, 31% between 0.1 and 1 year^{-1}, 26% between 1 and 10 year^{-1}, while 1% was fished with a frequency of 10–100 year^{-1}. A higher spatial resolution of 0.003 km^2 (1000 × 1000 spatial units per rectangle) would show that 86% of the rectangle is not fished, 5% was fished between 10 and 100 year^{-1}, while 9% was fished with a frequency of 100–1000 year^{-1}. Even though the proportions may change, at the smallest spatial scale (1000 × 1000 spatial units per rectangle) all rectangles only consist of a large part that is not fished and a smaller part that is fished with a frequency of more than 10 year^{-1} or even more than 100 year^{-1}. In contrast, a lower spatial resolution results in a more evenly distributed fishing pattern.

Increasing the temporal scale of this rectangle (effort = 50–100 days at sea, 30 × 30 spatial units per rectangle) from the 1-year period that was previously described to a 10-year period would show that now only 4% of the rectangle was not fished, 8% was fished less than 0.1 year^{-1}, 38% between 0.1 and 1 year^{-1}, 46% between 1 and 10 year^{-1}, while 4% was fished with a frequency of 10–100 year^{-1}. Thus, lowering the temporal resolution results in a more evenly distributed fishing pattern.

The perceived fishing impact on a population expressed as the annual mortality is determined by the spatial and temporal scale chosen (Fig. 4). Increasing the spatial scale affects mortality differently, depending on the amount of effort exerted on the rectangle. The low effort rectangles (0–10 days at sea per year) are least affected by the spatial scale, showing a mortality between 5% and 15%, while in the high effort rectangles (500–1000 days at sea per year) mortality increases to a maximum at a scale of 30 × 30 spatial units per rectangle, after which it decreases, until at a scale of 1000 × 1000 spatial units per rectangle, the annual mortality becomes independent of the sensitivity of the species to the gear. At that scale, both virtual species suffer 11% annual mortality in the low effort rectangles and 17% mortality in the high effort rectangles, in spite of being differently vulnerable to individual trawling events (25% vs. 75% mortality).

An increase of the temporal scale from 1 to 10 years (at a spatial scale 30 × 30 spatial units per rectangle) results in minor increase from 4% to 15% in annual mortality in the case of a species that is not very sensitive to the gear (25% mortality after one trawling event) and in a low effort rectangle. In contrast, it results in a markedly larger increase from 41% to 73% in annual mortality for a species that is sensitive to the gear (75% mortality after one trawling event) and in a high effort rectangle.

Discussion

This study clearly shows that the overall amount of effort and the choice of spatial and temporal scales determine our perception of fishing impact both in terms of the spatial distribution of fishing effort as well as estimated fishing-induced mortality.

The results show that at the smallest spatial scale (1000 × 1000 spatial units per rectangle, surface area of approximately 56 m × 56 m), the rectangle gets divided into a large area that is not trawled (and hence 0% mortality) and a smaller area that is trawled heavily with close to 100% annual mortality for all species that remain inside the spatial unit and are, even marginally, affected by the gear. For such sedentary species, the vulnerability to the gear hardly makes any difference, because in those spatial units where fishing occurs, the trawling intensity is so high that even

Fig. 1. Spatial distribution of Dutch beam trawl fleet in the southeastern North Sea at different spatial scales. Grey scales indicate the number of fishing registrations; fishing intensity increases from white to black. The 100 × 100 and 300 × 300 resolution examples are enlargements of the rectangle indicated in the 30 × 30 resolution. The 1000 × 1000 resolution example is an enlargement of the rectangle indicated in the 300 × 300 resolution. More information on these scales can be found in Table 1.

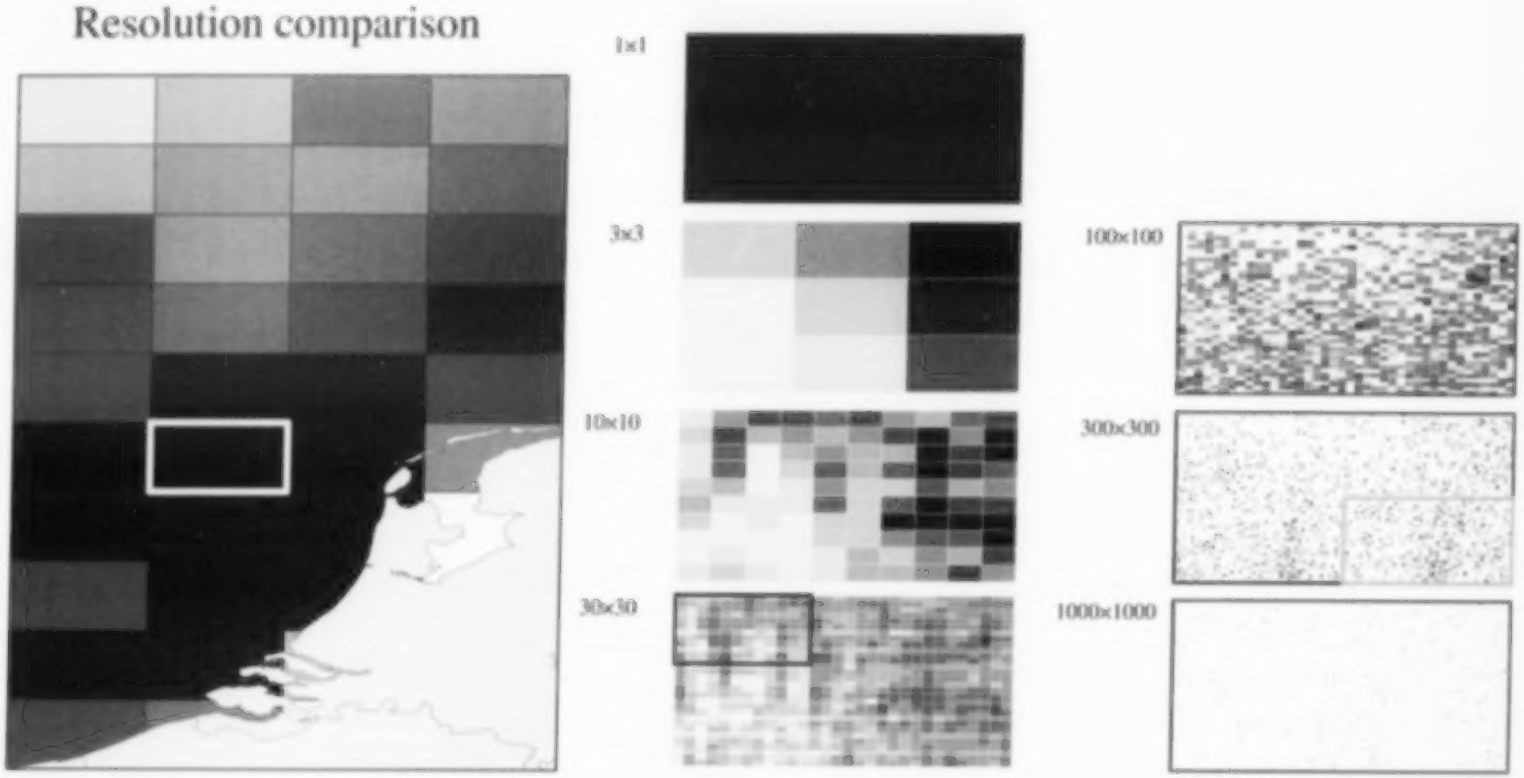

Resolution comparison
1x1
3x3
10x10
30x30
100x100
300x300
1000x1000

Fig. 2. Similarity in spatial distribution of the Dutch beam trawl fleet in the southeastern North Sea for the period 1994–2005 at different scales between years with varying interval as expressed by the Pearson's correlation coefficient (*r*).

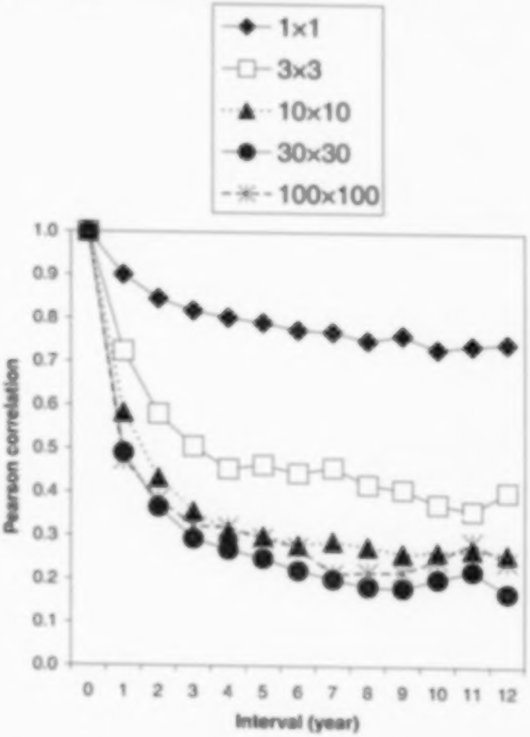
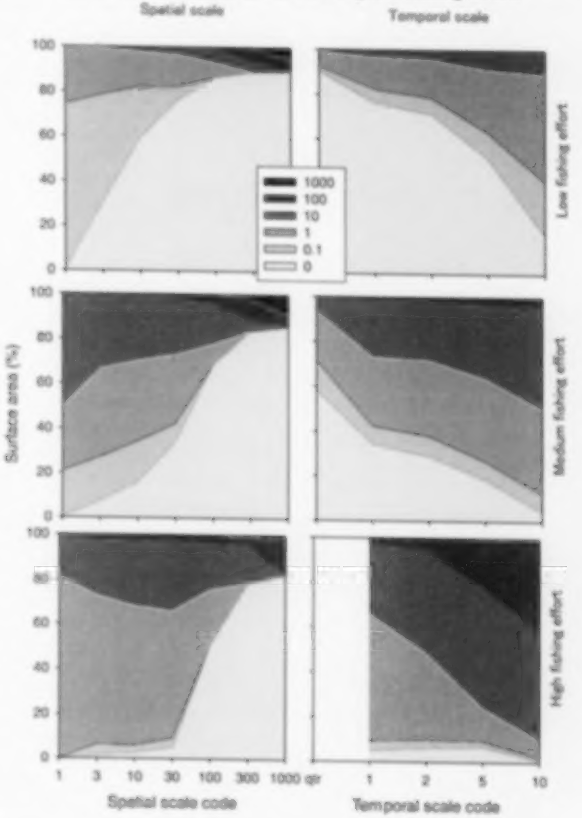


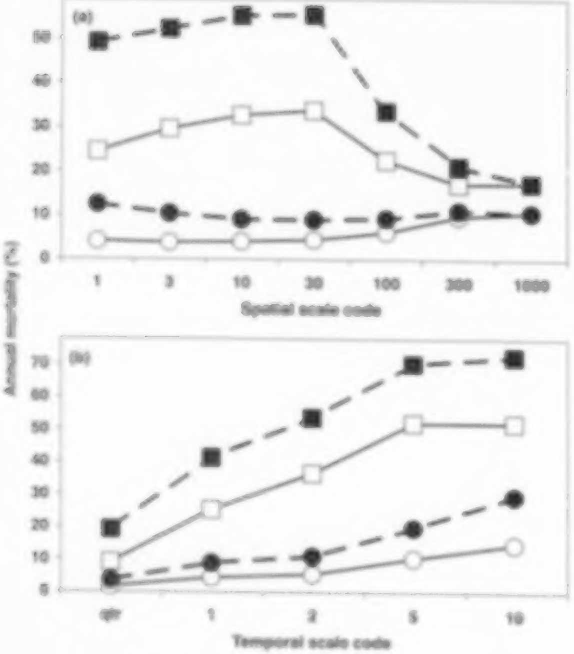
Fig. 3. Proportion of the area of an ICES rectangle trawled with a specific frequency (year^{-1}) depending on the fishing effort (low = 0–10, medium = 50–100, and high = 500–1000 days at sea per year) and spatial and temporal scales. The spatial and temporal scale codes are described in Tables 1 and 2, respectively. The effect of the spatial scale was assessed using a constant temporal scale of 1 year, while the effect of temporal scale was assessed at a constant spatial scale of 3.43 km^2. At a temporal scale equal to quarter (qtr), there were no ICES rectangles with the highest fishing effort.



very modest single-event mortalities of 5%–10% or higher result in an overall annual mortality of close to 100% in that spatial unit. Thus, for sedentary species that are vulnerable to the impact of a particular bottom trawl, the overall annual mortality in an ICES rectangle (or larger) is almost entirely determined by the proportions of trawled versus untrawled areas. For long-lived species, the effect of temporal scale becomes important because the results show that larger temporal scales (aggregation over more years) result in a more even distribution, thereby affecting the proportion of trawled versus untrawled areas and hence the overall annual mortality.

The consequences of spatial and temporal scale on the estimation of fishing impacts can best be understood with a few concrete examples. For a fish species such as plaice (*Pleuronectes platessa*), which is known to migrate over distances larger than ICES rectangles (Bolle et al. 2005), the coarsest resolution (i.e., ICES rectangle, code 1) is probably adequate, implying that in a high effort rectangle about 80% is fished with a frequency of 0.1–1 year^{-1} while the remaining 20% is fished with a frequency of 1–10 year^{-1}. Assuming a gear catches about 75% of the fish in its path, the annual mortality should be about 50%. As the scale of ICES rectangles is mostly used in studies aimed at estimating the fishing impact on fish species (e.g., Pope et al. 2000), these estimates are probably correct. In contrast, for most benthic invertebrate species, higher spatial resolutions between anywhere from 30 km^2 (code 10) to 3.4 km^2 (code 30) are used (e.g., Hiddink et al. 2006*a*, 2006*b*; Piet et al. 2000; Dinmore et al. 2003). For some mobile, epibenthic species, this scale may be appropriate, but for a sedentary mollusk like the ocean quahog (*Arctica islandica*), much smaller scales of 0.3 km^2 (code 100) or even 0.03 km^2 (code 300) are probably better. Assuming a low mortality of 25%, which is only slightly higher than the measured 16% (Lindeboom and de Groot 1998), then it becomes obvious that the choice of spatial scale makes a considerable difference; while the usual resolutions would show a 33%–34% annual mortality in a high effort rectangle, the scales of 0.3 and 0.03 km^2 would give annual mortalities of 22% and 17%, respectively. However, considering that the ocean quahog is a long-lived species (Witbaard et al. 1999), this reduction by half due to the choice of an appropriate spatial scale may be countered by a doubling of the annual mortality from 25% to 52% if the temporal scale is increased from 1 year to 10 years. Thus, as species become more sedentary,

Fig. 4. Spatial (*a*) and temporal (*b*) scales determine the annual mortality (%) in ICES rectangles with low (0–10 days at sea) and high (500–1000 days at sea) fishing effort and for species that differ in vulnerability, expressed in terms of the percent mortality caused by one trawling event. Open circles indicate low effort and 25% mortality; solid circles show low effort and 75% mortality; open squares represent high effort and 25% mortality; solid squares show high effort and 75% mortality.



even the mortality estimates based on (what are currently considered) high spatial resolution effort data may overestimate the actual mortality by more than a factor of 2. In contrast, annual mortality estimates of long-living species may be underestimated by a factor of 2 in studies that aggregate the data into yearly periods.

A problem with this analysis, however, is that it is based on VMS data that provide information on the location of a vessel at a certain interval but negates the fact that the position registrations represent actual tracks. The amount of fishing effort is essentially condensed in these positions, thereby incorrectly increasing the patchiness of the fishing activity and hence underestimating the estimated fishing impact. The main effect that the spatial resolution has on the mortality estimate appears to be determined by the fact that the proportion of unfished area increases. While in the low and medium effort rectangles this already becomes important at any resolution smaller than that of the ICES rectangle (codes > 1), for the high effort rectangles this only becomes important beyond code 30. This suggests that the strong effect of the unfished rectangles on the mortality estimate could be avoided if enough VMS positions were recorded or if this analysis was based on the actual track. If, indeed, the number of registrations is limiting the use of appropriate spatio-temporal scales, than this reinforces the statement of Hiddink et al. (2006*b*) that the constraints on compiling and accessing basic fishery data are an ongoing impediment to operationalizing an ecosystem approach to fisheries in the North Sea and other EU waters.

A fundamental goal in ecology is to determine the dynamic processes underlying observed patterns. The single greatest difficulty confronting this important objective is that any pattern detected, and ultimately the understanding of the underlying dynamic processes, depends on the spatial scale at which we make our observations (Wiens 1989; Levin 2000). The challenge is to identify the appropriate scales of observation for ecological investigation (Levin 1992). The various ecosystem components that are considered when determining the fishing impact on the marine ecosystem (e.g., habitat with possible structural elements, benthic species or fish) may all have different natural or characteristic scales that are optimal for describing the components' functioning and dynamic. These scales need to be identified for each of these components to assess their state, understand how it is impacted by human activity, and take appropriate measures to conserve it (Rand 1994; Bishop et al. 2002).

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Influences of prey- and predator-dependent processes on cadmium and methylmercury trophic transfer to mummichogs (*Fundulus heteroclitus*)

Daisuke Goto and William G. Wallace

Abstract: Factors affecting CH_3Hg and Cd trophic transfer from six invertebrates and a juvenile fish prey to mummichogs (*Fundulus heteroclitus*) were investigated using prey-dependent and predator-dependent approaches. Prey-specific trophic availability of metals to mummichogs was estimated using a subcellular partitioning approach (i.e., trophically available metals (TAM), as originally proposed by Wallace and Luoma (2003)). The proportions of CH_3Hg partitioned to TAM (CH_3Hg-TAM%) ranged from 39.7% to 82.7%, while the proportions of Cd partitioned to TAM (Cd-TAM%) ranged from 51.8% to 67.4%. CH_3Hg-TAM% and Cd-TAM% were significantly correlated with the proportions of metals released from prey by in vitro solubilization with mummichog gut fluid (i.e., gut solubilizable metals (GSM)), CH_3Hg-GSM% and Cd-GSM%, respectively. This relationship suggests that the majority of CH_3Hg and Cd partitioned as TAM in prey were solubilized in the digestive tract of mummichogs. Mummichogs retained 52.7% to 73.7% of the ingested CH_3Hg, which was comparable with prey-specific CH_3Hg-TAM% and CH_3Hg-GSM%. However, mummichogs retained only 3.2%–11.0% of ingested Cd, which was substantially lower than Cd-TAM% or Cd-GSM%. These results suggest that solubilizable, prey-associated CH_3Hg was transported through the gut membrane of mummichogs, whereas the majority of prey-associated Cd was excluded and subsequently eliminated, regardless of its subcellular partitioning.

Résumé : Nous avons étudié les facteurs qui affectent le transfert trophique de CH_3Hg et de Cd de six proies invertébrées et d'un jeune poisson proie au choquemort (*Fundulus heteroclitus*) en utilisant des approches dépendantes des proies et dépendantes des prédateurs. Nous avons estimé la disponibilité trophique des métaux aux choquemorts spécifique à chaque proie à l'aide de la méthode de partitionnement subcellulaire (c'est-à-dire les métaux trophiquement disponibles (TAM), méthode proposée au départ par Wallace et Luoma (2003)). Les proportions du CH_3Hg réparti en TAM (CH_3Hg-TAM%) varient de 39,7 % à 82,7 %, alors que les portions du Cd partitionné (Cd-TAM%) varient de 51,8 % à 67,4 %. Il y a une corrélation significative entre CH_3Hg-TAM% et Cd-TAM% et les proportions de métaux libérés des proies par une solubilisation in vitro avec du liquide provenant du tube digestif de choquemorts (c'est-à-dire, les métaux solubilisables dans le tube digestif (GSM)), respectivement CH_3Hg-GSM% et Cd-GSM%. Cette relation laisse croire que la plus grande partie du CH_3Hg et du Cd partitionnés en TAM chez les proies est solubilisée dans le tube digestif des choquemorts. Les choquemorts retiennent 52,7 % à 73,7 % du CH_3Hg ingéré, ce qui se compare aux CH_3Hg-TAM% et CH_3Hg-GSM% spécifiques aux proies. Cependant, les choquemorts ne retiennent que 3,2 % à 11,0 % du Cd ingéré, ce qui est considérablement plus bas que les valeurs de Cd-TAM% et Cd-GSM%. Ces résultats indiquent que le CH_3Hg solubilisable associé aux proies est transporté à travers la membrane du tube digestif des choquemorts, alors que la plus grande partie du Cd associé aux proies est exclue et ensuite éliminée, indépendamment de son partitionnement subcellulaire.

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Introduction

Trophic transfer processes are an integral part in understanding the fates and biogeochemical cycling of trace metals in coastal marine ecosystems (Fisher and Reinfelder 1995). Due to potential bioaccumulation and subsequent toxic effects in organisms, predicting the trophic transfer efficiency of trace metals in coastal marine ecosystems is of great interest to applied ecologists and environmental scientists.

Although aquatic predators are exposed to trace metals through a variety of sources (i.e., sediments, water, and diet), the predominant source of many metals for these predators is the diet (Hall et al. 1997; Wang 2002; Luoma and Rainbow 2005). Furthermore, studies have shown that dietary exposure to metals is more likely to cause toxic effects in fish than aqueous exposures (Woodward et al. 1994,

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1995; Farag et al. 1999). Many deposit-feeding macroinvertebrates are constantly in direct contact with sediments (the main source of metals in benthic food chains) and are also a major component of the diet of various benthivorous predators in coastal environments (Kneib 1986; Steimle 1994; Carlson et al. 1997). Understanding the extent of and processes involved in metal bioaccumulation in benthic macroinvertebrates is thus essential to accurately predict metal trophic transfer to and associated ecological impacts on higher trophic-level predators in coastal environments.

Despite a number of studies conducted on metal trophic transfer in aquatic ecosystems, there are still uncertainties in predicting food chain effects of bioavailable metals in the natural environment. For the past decade, there has been a growing body of evidence suggesting that intracellular partitioning of metals in prey can influence metal trophic transfer to predators (e.g., Nott and Nicolaidou 1994; Wallace et al. 1998; Rainbow et al. 2007). The relevance of intracellular metal distributions in lower trophic-level organisms to metal trophic transfer results from differential bioavailabilities of metal sequestered among various subcellular components (Wallace and Lopez 1997; Nott 1998; Zhang and Wang 2006). A series of experimental studies have demonstrated a direct 1:1 relationship between metals partitioned to cytoplasm in dietary sources (phytoplankton) and metals assimilated by herbivorous marine invertebrates with simple digestive strategies (e.g., copepods and bivalve larvae) (Reinfelder and Fisher 1991; Hutchins et al. 1995; Mason et al. 1995). This direct relationship is, however, less evident in other herbivores with more complex digestive processes, such as adult bivalves, which appear to be able to extract metals from noncytoplasmic compartments (Wang and Fisher 1996; Reinfelder et al. 1997). Furthermore, more recent studies have shown that the relationship between cytoplasm-associated metals in phytoplankton and metal assimilation by herbivores is highly species- as well as metal-specific (Xu et al. 2001; Schlekat et al. 2002; Ng et al. 2005).

A study by Wallace and Lopez (1997) has further demonstrated that in addition to cytoplasm-bound metals, omnivorous decapods are also able extract metals partitioned to organelles in deposit-feeding oligochaetes. Because of the high bioavailability of metals bound to these subcellular compartments to consumers, Wallace and Luoma (2003) have proposed that metals associated with cytosolic proteins and organelles can be compartmentalized as trophically available metal (TAM; i.e., metals readily available to predators). These studies have suggested that there is a 1:1 relationship between the percentage of cadminum (Cd) bound to the proposed TAM compartment of prey and the percentage assimilated by omnivorous decapods (Wallace and Luoma 2003). More recent studies have, however, shown that this direct relationship between metals associated with the TAM compartment of prey and metal assimilation efficiency (AE) by predators is not applicable to many aquatic organisms (e.g., carnivorous gastropods and fish) (e.g., Cheung and Wang 2005; Rainbow et al. 2006, 2007). Furthermore, some predators also seem to be able to partially solubilize and assimilate metals partitioned to non-TAM compartments (e.g., mineralized granules) (Cheung and Wang 2005; Zhang and Wang 2006).

In addition to intracellular metal storage in prey, digestive processes (e.g., solubilization and membrane transport) in predators are also likely to influence dietary metal bioavailability (Farmanfarmaian 1985; Leaner and Mason 2002; Rainbow et al. 2006), especially in higher trophic-level predators (Hall et al. 1997; Luoma and Rainbow 2005). Previous studies have suggested that differential metal assimilation of compartmentalized metals is possibly caused by species-specific digestive physiology (Amiard-Triquet et al. 2006; Rainbow et al. 2006, 2007). If, as previous studies suggest, metals sequestered to the proposed TAM compartment of prey are solubilized and absorbed by a predator, intracellular partitioning of metals within prey could, alone, predict the extent of dietary metal bioavailability to predators (Wallace and Luoma 2003; Seebaugh and Wallace 2004; Seebaugh et al. 2005). There is, however, only a limited understanding of how predator's digestive processes can affect metal bioavailability from ingested prey. The relationship between intracellular partitioning of metals (i.e., sequestration to the TAM compartment) in prey and metal solubilization in a predator's digestive tract prior to absorption is, therefore, not clearly understood (Wang 2002).

Since the digestive system of many carnivores is generally different from that of herbivores (Clearwater et al. 2005; Lopez 2005), the digestive processes of these predators may have different effects on the extent of metals assimilated from ingested prey. Recent studies have shown that the bioavailability of some metals from dietary sources can be estimated by an in vitro solubilization technique using gut fluids extracted from marine organisms (e.g., Mayer et al. 1996; Leaner and Mason 2002; Weston et al. 2004). While the application of the in vitro solubilization approach to the estimation of metal bioavailability has mainly been investigated with deposit-feeding polychaetes (Chen and Mayer 1999; Chen et al. 2002; Weston et al. 2004), a few studies have investigated the applicability of this approach with other trophic groups, including carnivorous fish (Fair and Sick 1984; Leaner and Mason 2002).

The objective of the present study was to examine the mechanisms involved in metal trophic transfer to predators, specifically how prey-dependent (i.e., subcellular compartmentalization in prey) and predator-dependent (i.e., solubilization in the digestive tract of a predator) processes interactively influence trophic transfer (i.e., AE in a predator) of Cd and methylmercury (CH_3Hg). While Cd and CH_3Hg are both widespread in freshwater and marine ecosystems and often bioaccumulate in fish, these metals behave differently in food webs (bioreduction vs. biomagnification, respectively; Wang 2002).

In this study, mummichogs (*Fundulus heteroclitus*), an abundant resident fish in tidal creeks and salt marshes of the northwest Atlantic coast (Nixon and Oviatt 1973; Valiela et al. 1977; Meredith and Lotrich 1979), were used as a predator. Mummichogs play an essential role as an intermediate predator linking sediment-associated invertebrates (and therefore sediment-associated trace metals) to piscivorous fishes and wading birds (Peters and Schaaf 1991; Nemerson and Able 2003). Understanding what determines the extent of metal trophic transfer to mummichogs would thus provide much needed information on the potential for ecological impacts of trace metals on coastal marine ecosystems.

Materials and methods

Field sampling and sample handling

Large adult mummichogs (70–90 mm in total length) used for laboratory feeding experiments and gut fluid extraction were collected from Tuckerton, New Jersey, USA, using minnow traps. Prey species in this study were chosen to represent prey items commonly observed in the guts of a variety of coastal benthic predators, including mummichogs (Kneib et al. 1980; Clements and Livingston 1983; Weis 2005). *Palaemonetes pugio* (grass shrimp, 20–40 mm in total length) and *Gammarus mucronatus* (amphipod) were collected from Staten Island, New York, USA. Mummichogs, grass shrimp, and amphipods were then acclimated to laboratory conditions (20 °C, 15‰ filtered seawater) and fed with a commercial fish food (TetraMin) for at least 30 days prior to the experiments. *Neanthes virens* (clamworm), *Leptocheirus plumulosus* (amphipod), *Chironomus dilutus* (aquatic insect) larva, and juvenile *Cyprinodon variegatus* (sheepshead minnow, <30 days) were obtained from Aquatic Research Organisms, Inc. (Hampton, New Hampshire, USA). *Leptocheirus plumulosus* and *Cyprinodon variegatus* were acclimated in 20 °C, 15‰ filtered seawater and fed on commercial fish food prior to the experiments. *Chironomus dilutus* was maintained in 2.5‰ filtered seawater. *Neanthes virens* were maintained in sieved sediments (>500 µm) collected from Staten Island, New York, USA.

Radiolabeling of prey organisms

Radioisotopes (^{109}Cd and ^{203}Hg) were obtained from New England Nuclear, Boston, Massachusetts, USA, and $CH_3{}^{203}Hg$ was synthesized using a method by Rouleau and Block (1997). Prior to radiolabeling, all prey organisms were depurated for 24 h in filtered 15‰ seawater, except *Chironomus dilutus*, which was depurated in filtered 2.5‰ seawater. Prey organisms were exposed to trace amounts of the radioisotopes (^{109}Cd and $CH_3{}^{203}Hg$) in 1.5 or 3 L (depending on prey biomass) of 0.45 µm filtered 15‰ (or 2.5‰ for *Chironomus dilutus*) seawater at 20 °C for 96 h. Specific radioisotope spike concentrations were based on the prey-specific preliminary experiments and ranged from 54.4 to 433.5 kBq·L^{-1} for ^{109}Cd and from 1.69 to 4.75 kBq·L^{-1} for $CH_3{}^{203}Hg$. Increases in acidity due to ^{109}Cd spikes were offset by the addition of appropriate volumes of 0.5 mol·L^{-1} NaOH to maintain a pH in the exposure solutions of ~7–8 (Blackmore and Wang 2004). Following exposure, prey organisms were rinsed with filtered sea water twice and then assayed for radioactivity with a gamma counter (Wallac 1480 Wizard gamma counter). Each type of radiolabeled prey was then randomly distributed among the following three tasks (except *Neanthes virens*, which were first cut into small pieces after decapitation): (*i*) feeding experiments, (*ii*) isolation of TAM, and (*iii*) in vitro gut solubilization of metals. Prey were then stored at –80 °C.

Determination of metal AE in mummichogs

AE of Cd and CH_3Hg in mummichogs were estimated through a series of pulse-chase feeding experiments (Wang and Fisher 1999). Previously radiolabeled prey were fed to mummichogs (70–80 mm; $n = 6$ per prey species). Feeding experiments were conducted in 38 L glass aquaria filled with aerated, filtered 15‰ seawater at room temperature. Mummichogs were anesthetized with 150 mg·L^{-1} MS-222 (tricaine methanesulfonate) in filtered 15‰ seawater for 5 min, force-fed prey, and were immediately assayed for radioactivity with a gamma counter (Rouleau et al. 1998). This activity was set as the initial radioactivity ($t = 0$), representing 100% of ingested metals. Following this initial radioanalysis, each fish was transferred to depuration aquaria (38 L) where they were individually housed in 11.5 cm × 11.5 cm × 10.5 cm chambers (Super Breeder, International Pet Supplies and Distribution, Inc., San Diego, California, USA) and fed with commercial freeze-dried krill (a ration of 5% of fish body weight per day). To prevent cycling of excreted radioisotopes, aquaria water was filtered through submersible power filters at ~200 L·h^{-1} flow (Duetto 50, Aquarium Systems, Inc., Mentor, Ohio, USA), and fecal material was physically separated from fish within each chamber. AEs of ingested metals were calculated at $t = 24$ h using the ratio of retained isotope activity at $t = 24$ h to the activity at $t = 0$ h (Wang and Wong 2003; Rainbow et al. 2006).

Isolation of TAM in prey

The fraction of metals associated with the TAM compartment, as originally proposed by Wallace and Luoma (2003), was isolated from radiolabeled prey organisms using cellular fractionation techniques. First, previously frozen prey organisms ($n = 6$ per prey species; each replicate consisted of a composite of ~500 mg wet tissue specimens) were thawed on ice, dabbed dry, and weighed; short-term freezing appears to have no impact on subcellular metal distributions (Pan and Wang 2008). Animals were then radioanalyzed with a gamma counter. Tissues were then transferred into preweighed centrifuge tubes and homogenized with a Polytron tissue homogenizer (Kinematica, Luzern, Switzerland) in cold 20 mmol·L^{-1} TRIS buffer (pH adjusted to 7.6) (Wallace et al. 2003). The homogenates were fractionated by centrifugation at 1450*g* for 15 min, producing a pellet containing tissue fragments and other cellular debris (including exoskeleton of the crustacean prey) and a supernate containing cytosolic proteins and organelles (i.e., TAM) (Wallace and Luoma 2003). Each fraction was immediately radioassayed with a gamma counter, as described below.

Gut fluid extraction from mummichogs

Digestive fluid secretion in mummichogs was induced by feeding mealworms for 30 min. Fish were then euthanized with an overdose of MS-222, and the entire intestine was immediately excised. Gut contents were then transferred to a 1.5 mL centrifuge tube and centrifuged at 8000*g* for 30 min (Leaner and Mason 2002). Supernates (i.e., gut fluids) were collected and stored at –80 °C until use in the in vitro solubilization experiments (there is no known effect of freezing on metal solubilization; Leaner and Mason 2002). The total volume of gut fluids collected from 100 mummichogs was ~15 mL (= 0.15 mL·fish^{-1}).

In vitro solubilization of metals from prey

The effects of mummichog digestive processes on metal bioavailability from prey were estimated by an in vitro solubilization technique (Mayer et al. 1996; Leaner and Mason 2002), in which radiolabeled prey was incubated in vitro with extracted mummichog gut fluid. Mummichog gut fluid was thawed and pH was adjusted to 8.5 with 0.5 mol·L^{-1} NaOH (during food digestion, the pH of mummichog gut fluid is normally in the range of 8 to 9; Nicholls 1933). The incubation was started by adding gut fluid to thawed prey tissues (100 to 500 mg wet weight·replicate^{-1}) in a 1.5 mL centrifuge tube (a ratio of wet weight of prey tissues (g):volume of mummichog gut fluid (mL) = 1:5). The mixture of prey tissues and mummichog gut fluid were incubated at room temperature (~20 °C) on an orbital shaker at 500 rpm for 7 h (the approximate gut retention time of mummichogs at 20 °C; Nicholls 1933).

Incubations were terminated by heating samples at 100 °C in a water bath for 1 min. Upon termination, samples were radioanalyzed by a gamma counter. Samples were then centrifuged with a tabletop centrifuge (Eppendorf 5415C) at 8000*g* for 30 min to isolate the solubilized fraction of metals (i.e., the supernate) (Leaner and Mason 2002) from the insoluble fraction (i.e., the pellet). Pellets were rinsed with 0.4 mL of 20 mmol·L^{-1} TRIS buffer (pH adjusted to ~7.5) and centrifuged again at 8000*g* for 30 min, which was repeated twice (Ahrens et al. 2001). All the resultant supernates were combined in one 1.5 mL centrifuge tube. Supernates and pellets were then radioanalyzed, and the percentage of gut solubilizable metals (GSM) (i.e., (metals in a supernate)/(total metals in prey) × 100) was calculated.

Radioanalysis and ^{109}Cd and CH$_3$^{203}Hg body burden calculations

The radioactivity of samples (whole body and subcellular fractions) was determined with a Wallac 1480 Wizard gamma counter equipped with a 3 inch (1 inch = 2.54 cm) well type NaI crystal detector. Photon emissions of ^{109}Cd and CH$_3$^{203}Hg were determined at 88 and 279 keV, respectively. Propagated counting errors were maintained at or below 5%. The raw data, cpm (counts per minute), were converted to dpm (disintegration per minute) using appropriate standards and half-life corrections. Percent subcellular distributions of ^{109}Cd and CH$_3$^{203}Hg within animal tissues were calculated based on radioactivity recovered after homogenization. Based on previous studies, recovery of homogenate radioactivity subsequent to fractionation is consistently high (>85%) (i.e., (summation of radioactivity in each subcellular fraction)/(radioactivity following homogenization); Wallace et al. 1998; Wallace and Luoma 2003).

Statistical analyses

The normality of data was tested with Shapiro–Wilk's *W* test. The homogeneity of variance was tested with Levene's test. Data (percentages) for TAM fraction in prey, GSM fraction in prey, and AE of metals in mummichogs were arcsine-transformed prior to statistical analyses. As all variables were subject to measurement error, the relationships between (*i*) TAM and AE, (*ii*) GSM and AE, and (*iii*) TAM and GSM were analyzed with reduced major axis (RMA) regression (Sokal and Rohlf 1995). Differences among TAM, GSM, and AE were analyzed with one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) test. All statistical analyses were performed using STATISTICA version 7.1 (StatSoft, Inc., Tulsa, Oklahoma).

Results

AE of metals in mummichogs

There was no significant loss of either metal from mummichogs ingesting any of the prey species between 24 and 48 h points (Fig. 1, *t* test, $p > 0.05$). Therefore, AEs of both Cd and CH$_3$Hg from all prey species were determined at 24 h. At 24 h following the ingestion of prey, the retention of CH$_3$Hg by mummichogs was considerably higher (as much as ~20-fold) than that of Cd for all prey species (Fig. 1). Mummichogs retained 51.7% ± 4.3% (mean ± standard error, SE) of CH$_3$Hg from *Palaemonetes pugio*, 67.4% ± 1.1% from *Neanthes virens*, 59.5% ± 2.7% from *Cyprinodon variegatus*, 63.3% ± 1.8% from *Gammarus mucronatus*, 73.7% ± 4.5% from *Leptocheirus plumulosus*, and 89.3% ± 4.0% from *Chironomus dilutes* (Fig. 1).

In contrast, mummichogs retained only 6.8% ± 1.9% (mean ± SE) of Cd from *Palaemonetes pugio*, 3.2% ± 0.3% from *Neanthes virens*, 11.0% ± 2.2% from *Cyprinodon variegatus*, 6.9% ± 0.8% from *Gammarus mucronatus*, 9.3% ± 3.3% from *Leptocheirus plumulosus*, and 6.2% ± 1.1% from *Chironomus dilutes* (Fig. 1).

TAM in prey and its relationship to metal AE in mummichogs

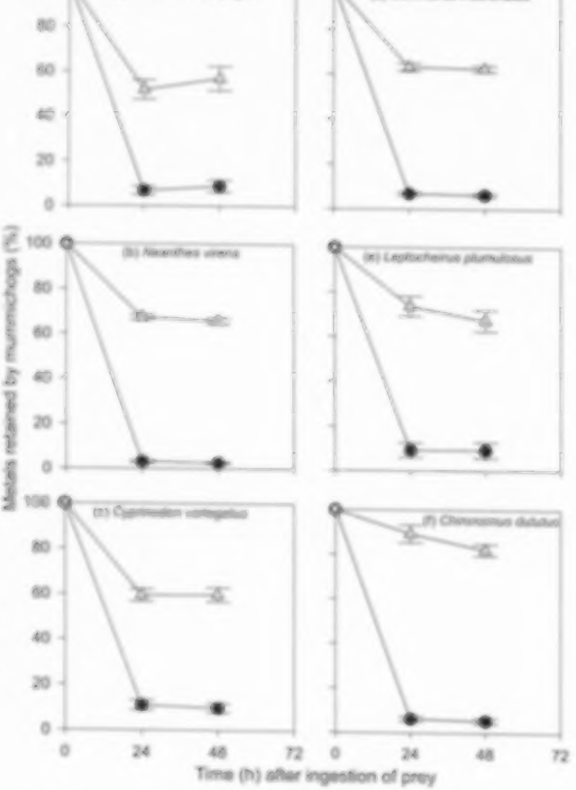
Intracellular partitioning of metals (i.e., TAM) within each prey species was highly species- as well as metal-specific. Compared with CH$_3$Hg, Cd partitioned to TAM (Cd-TAM) was considerably varied among prey species (Fig. 2). The proportion of Cd-TAM was highest in *Neanthes virens* (82.7% ± 1.0%, mean ± SE), which was followed by *Chironomus dilutes* (71.4% ± 2.3%), *Palaemonetes pugio* (67.5% ± 2.6%), *Leptocheirus plumulosus* (62.5% ± 1.5%), *Gammarus mucronatus* (47.4% ± 4.3%), and *Cyprinodon variegatus* (39.7% ± 3.0%) (Fig. 2).

In contrast, the proportion of CH$_3$Hg-TAM was fairly similar among prey species: 61.2% ± 1.3% (mean ± SE) in *Palaemonetes pugio*, 63.3% ± 1.8% in *Neanthes virens*, 51.8% ± 3.5% in *Cyprinodon variegatus*, 59.5% ± 2.8% in *Gammarus mucronatus*, 59.1% ± 1.2% from *Leptocheirus plumulosus*, and 58.3% ± 2.3% from *Chironomus dilutes* (Fig. 2). No relationship between TAM and AE for either metal was found ($p > 0.05$). Despite the high variability in Cd-TAM% among prey species, Cd-AE% remained fairly constant (~5%–10%) (Fig. 2). Additionally, although CH$_3$Hg-AE% was generally similar to CH$_3$Hg-TAM%, there was no relationship between these variables (Fig. 2).

GSM from prey and its relationship to metal AE in mummichogs

The proportions of metals released from prey by in vitro solubilization with mummichog gut fluid were considerably varied among prey species, especially for Cd (Fig. 3). A substantial proportion of Cd incorporated in prey was solubilized by gut fluid (i.e., GSM). More than 80% of Cd in *Neanthes virens* (94.4% ± 1.3%, mean ± SE) and *Chironomus dilutes* (84.5% ± 1.4%) was released by the end of incubation period (7 h) (Fig. 3). Compared with these invertebrates, a slightly lower proportion of Cd (~70%) was solubilized from crustaceans: *Leptocheirus plumulosus* (69.2% ± 2.9%), *Gammarus mucronatus* (68.2% ± 3.3%),

Fig. 1. Percentages of ^{109}Cd (circles) and CH$_3$^{203}Hg (triangles) retained in mummichogs (*Fundulus heteroclitus*) after 24 and 48 h following pulse-feeding of radiolabeled (*a*) *Palaemonetes pugio*, (*b*) *Neanthes virens*, (*c*) *Cyprinodon variegatus*, (*d*) *Gammarus mucronatus*, (*e*) *Leptocheirus plumulosus*, and (*f*) *Chironomus dilutes*. Error bars indicate ±1 standard error (SE); n = 6 for each prey item.



and *Palaemonetes pugio* (67.7% ± 4.8%), whereas a substantially lower proportion of Cd was released from *Cyprinodon variegatus* (32.4% ± 3.2%) (Fig. 3).

Compared with Cd, a slightly less proportion of CH$_3$Hg was solubilized from *Neanthes virens* (82.1% ± 1.3%) (Fig. 3). CH$_3$Hg-GSM% in amphipods and midge larvae (39.6% ± 4.5% from *Gammarus mucronatus*, 47.4% ± 1.9% from *Leptocheirus plumulosus*, and 64.9% ± 3.8% from *Chironomus dilutes*) were all considerably lower than the corresponding Cd-GSM% (t test, $p < 0.05$) (Fig. 3). CH$_3$Hg-GSM% in *Palaemonetes pugio* (61.7% ± 2.1%) was similar to Cd-GSM%, whereas CH$_3$Hg-GSM% in *Cyprinodon variegatus* (68.7% ± 2.9%) was substantially higher than Cd-GSM% (t test, $p < 0.05$) (Fig. 3). No relationship between GSM and AE was observed for either Cd or CH$_3$Hg (Fig. 3).

Relationship between TAM in prey and GSM from prey

There was a significant relationship between TAM% and GSM% (Fig. 4). When the data for both Cd and CH$_3$Hg were analyzed together with RMA regression, a moderate proportion of the variance (38.2%) in metal digestive solubility with mummichog gut fluid (GSM%) was explained by intracellular partitioning in prey (TAM%) (GSM = 0.956 TAM + 2.96, $p < 0.05$) (Fig. 4). When the data were analyzed for Cd only, the variance explained by RMA re-

Fig. 2. Relationship between percentages of ^{109}Cd (circles) and CH$_3$^{203}Hg (triangles) assimilated in mummichogs and percentages of ^{109}Cd and CH$_3$^{203}Hg associated with trophically available metals (TAM) (TAM-^{109}Cd% or TAM-CH$_3$^{203}Hg%) in *Palaemonetes pugio* (*Pp*), *Neanthes virens* (*Nv*), *Cyprinodon variegatus* (*Cv*), *Gammarus mucronatus* (*Gm*), *Leptocheirus plumulosus* (*Lp*), and *Chironomus dilutes* (*Cd*). Error bars indicate ±1 standard error (SE); broken line indicates a 1:1 relationship.

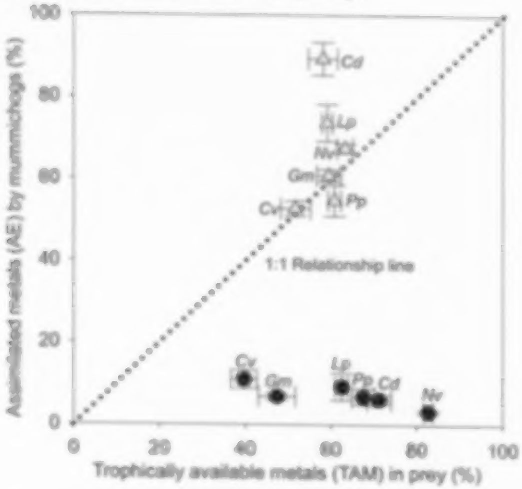
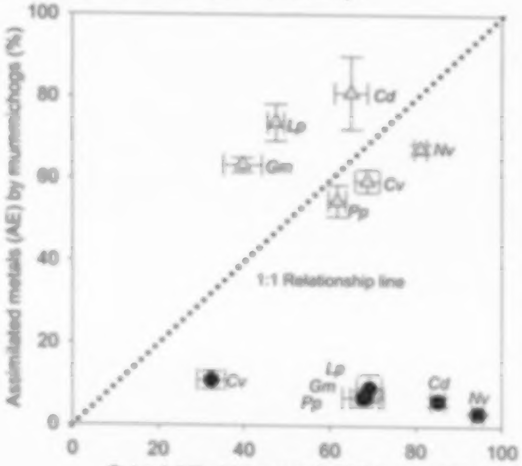


Fig. 3. Relationship between percentages of ^{109}Cd (circles) and CH$_3$^{203}Hg (triangles) assimilated in mummichogs and percentages of ^{109}Cd and CH$_3$^{203}Hg solubilized in vitro with mummichog gut fluid (GSM-^{109}Cd% or GSM-CH$_3$^{203}Hg%) in *Palaemonetes pugio* (*Pp*), *Neanthes virens* (*Nv*), *Cyprinodon variegatus* (*Cv*), *Gammarus mucronatus* (*Gm*), *Leptocheirus plumulosus* (*Lp*), and *Chironomus dilutes* (*Cd*). Error bars indicate ±1 standard error (SE); broken line indicates a 1:1 relationship.



gression (GSM = 0.938 TAM + 11.30, $p < 0.05$) increased to 57.3%, while the relationship between TAM% and GSM% for the CH_3Hg data alone was not significant ($p > 0.05$).

Species-specific comparisons of potential metal bioavailability and trophic transfer

A species-specific examination of potential metal bioavailability and trophic transfer estimated with the three different approaches (intracellular storage of metals in prey (TAM), digestive solubilization with mummichog gut fluid (GSM), and metal assimilation in mummichogs (AE)) showed a consistent and striking difference between Cd and CH_3Hg (Figs. 5 and 6). Overall, Cd-TAM% was comparable with that of Cd-GSM% in prey, though Cd-GSM% in some prey species (*Gammarus mucronatus*, *Neanthes virens*, and *Chironomus dilutes*) was slightly higher than Cd-TAM% (Fig. 5, Tukey's HSD test, $p < 0.05$). However, Cd-AE% was substantially lower than both Cd-TAM% and Cd-GSM% for mummichogs that were fed all prey types (Fig. 5, Tukey's HSD test, $p < 0.05$).

CH_3Hg-GSM% of *Neanthes virens* and *Cyprinodon variegatus* were slightly higher than CH_3Hg-TAM% (Fig. 6, Tukey's HSD test, $p < 0.05$), whereas in other prey species, CH_3Hg-GSM% was comparable with CH_3Hg-TAM%. In contrast with Cd, CH_3Hg-AE% was generally comparable with (for *Palaemonetes pugio* and *Cyprinodon variegatus*), or slightly higher (for *Gammarus mucronatus*, *Leptocheirus plumulosus*, *Neanthes virens*, and *Chironomus dilutes*) than either CH_3Hg-TAM% or CH_3Hg-GSM% in all prey species (Fig. 6, Tukey's HSD test, $p < 0.05$).

Discussion

Effect of metal intracellular partitioning within prey on metal bioavailability to predators

The trophic availability of trace metals in prey estimated from intracellular compartmentalization is often highly metal- as well as species-specific (Ng et al. 2005; Zhang and Wang 2006; Rainbow et al. 2007). It has been suggested that differential trophic availability of metals may be influenced by metal-handling capability of prey (Wallace and Luoma 2003). High percentage of Cd partitioned to a soluble intracellular fraction (e.g., cytosol) in marine invertebrates is often observed in many studies (Seebaugh et al. 2005; Rainbow et al. 2006; Zhang and Wang 2006). This partitioning pattern is likely due to strong affinity to high sulphur content metal-binding proteins (e.g., metallothioneins) in the soluble fraction (Bebianno and Langston 1991, 1992). In the present study, a relatively wide range of Cd partitioning (40%–80%) to the TAM compartment in prey species was observed. However, Cd-AE values in mummichogs were not influenced by this partitioning (AE = 3%–11%). Mummichogs assimilated only a small fraction of Cd in prey, which is comparable with a study by Seebaugh et al. (2005) with mummichogs feeding on grass shrimp (68% to 69% Cd-TAM in shrimp vs. 3% to 19% Cd-AE in fish). Similar results have also been documented for other fishes: *Cyprinus carpio* feeding on oligochaetes (72% Cd-TAM in oligochaetes vs. 9.8% Cd-AE in fish; Steen Redeker et al. 2007), *Oncorhynchus mykiss* feeding on oligochaetes (72% to 80% Cd-TAM in oligochaetes vs. 0.9% to 6.4% Cd-AE in fish; Ng and Wood 2008), and *Terapon jarbua* feeding on a variety of prey species (40% to 90% Cd-TAM vs. 3% to 9% Cd-AE; Zhang and Wang 2006). In these studies, despite the high percentages of Cd-TAM in prey, these fishes had low Cd-AE values. Low assimilation of Cd by fish has been frequently observed in many studies (e.g., Pentreath 1977; Harrison and Klaverkamp 1989; Baines et al. 2002). These studies have demonstrated that unlike invertebrates, regardless of the amount of Cd ingested, both freshwater and marine fishes do not take up a substantial amount of Cd into their tissues. Based on these findings, it is highly unlikely that intracellular partitioning of Cd with prey species plays a major role controlling its ultimate trophic availability to carnivorous fish.

Although high AE of dietary CH_3Hg (generally >80%) in fish is commonly observed in many studies (e.g., Rodgers and Beamish 1982; Rouleau et al. 1998; Wang and Wong 2003), the underlying mechanisms have not been fully explored. Furthermore, compared with Cd, fewer studies have been conducted on CH_3Hg partitioning in dietary sources and its relationship with CH_3Hg assimilation in consumers. Several studies have demonstrated that the proportion of CH_3Hg partitioned to cytoplasm in phytoplankton is generally comparable with CH_3Hg AE in herbivores such as copepods and amphipods (Mason et al. 1995, 1996; Lawson and Mason 1998). In the present study, CH_3Hg AE values in mummichogs were considerably varied (50%–90%) among prey species (decapods, amphipods, polychaetes, midge larvae, and juvenile fish), while the proportions of CH_3Hg-TAM associated with these prey surprisingly varied little

Fig. 4. Relationship between percentages of ^{109}Cd (circles) and $CH_3{}^{203}Hg$ (triangles) solubilized in vitro with mummichog gut fluid (GSM-^{109}Cd% or GSM-$CH_3{}^{203}Hg$%) from various prey and percentages of ^{109}Cd and $CH_3{}^{203}Hg$ associated with trophically available metals (TAM) (TAM-^{109}Cd % or TAM-$CH_3{}^{203}Hg$%) in the same prey: *Palaemonetes pugio* (*Pp*), *Neanthes virens* (*Nv*), *Cyprinodon variegatus* (*Cv*), *Gammarus mucronatus* (*Gm*), *Leptocheirus plumulosus* (*Lp*), and *Chironomus dilutes* (*Cd*). Error bars indicate ±1 standard error (SE); broken line indicates a 1:1 relationship; and solid line indicates the reduced major axis regression.

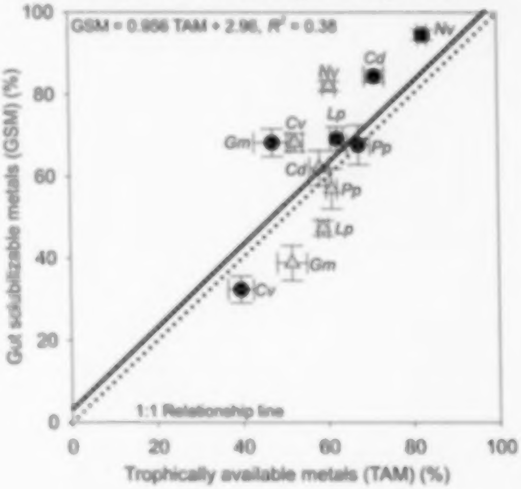


Fig. 5. Stages of Cd trophic transfer process: comparisons of percentages of ^{109}Cd associated with trophically available metals (TAM) (TAM-^{109}Cd%), percentages of ^{109}Cd solubilized in vitro with mummichog gut fluid (GSM-^{109}Cd%) from various prey, and percentages of ^{109}Cd assimilated in mummichogs feeding on the same prey: (*a*) *Palaemonetes pugio*, (*b*) *Neanthes virens*, (*c*) *Cyprinodon variegatus*, (*d*) *Gammarus mucronatus*, (*e*) *Leptocheirus plumulosus*, and (*f*) *Chironomus dilutes*. Error bars indicate ±1 standard error (SE); different letters above each bar indicate a significant difference (one-way analysis of variance followed by Tukey's honestly significant difference test; $p < 0.05$).

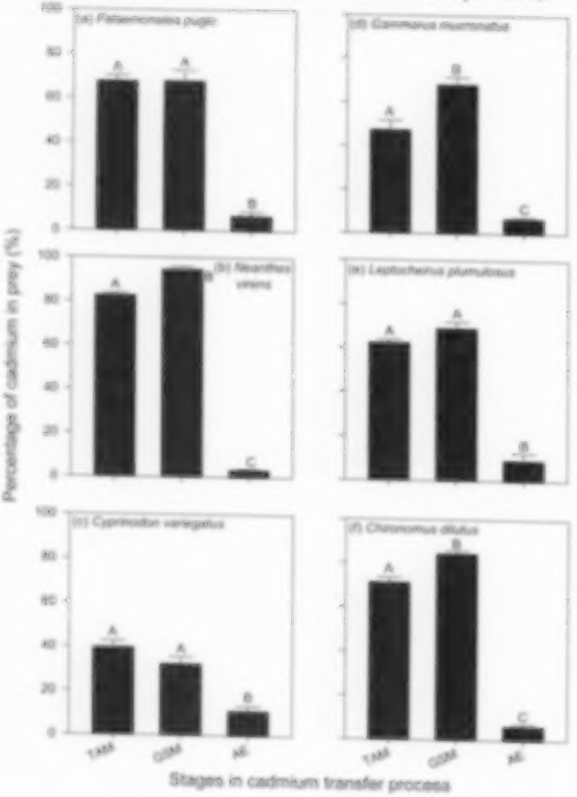
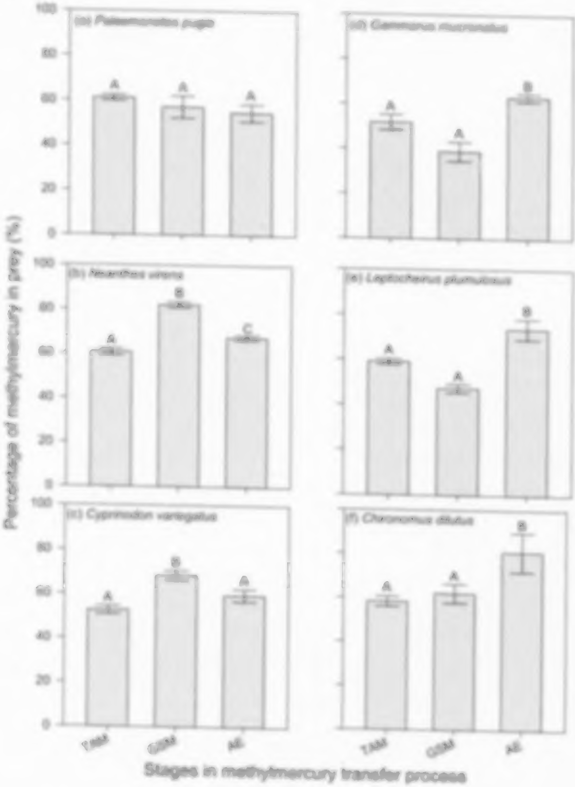


Fig. 6. Stages of CH$_3$Hg trophic transfer process: comparisons of percentages of CH$_3$^{203}Hg associated with trophically available metals (TAM) (TAM- CH$_3$^{203}Hg%), percentages of CH$_3$^{203}Hg solubilized in vitro with mummichog gut fluid (GSM- CH$_3$^{203}Hg%) from various prey, and percentages of CH$_3$^{203}Hg assimilated in mummichogs feeding on the same prey: (*a*) *Palaemonetes pugio*, (*b*) *Neanthes virens*, (*c*) *Cyprinodon variegatus*, (*d*) *Gammarus mucronatus*, (*e*) *Leptocheirus plumulosus*, and (*f*) *Chironomus dilutes*. Error bars indicate ±1 standard error (SE); different letters above each bar indicate a significant difference (one-way analysis of variance followed by Tukey's honestly significant difference test; $p < 0.05$).



(~60%). These findings suggest that the discrepancy in CH$_3$Hg trophic transfer may be due to the differences in digestive physiology among trophic groups. Herbivorous invertebrates with a simple digestive physiology (e.g., copepods) may be able to assimilate only CH$_3$Hg in the soluble fraction in the diet (Mason et al. 1995, 1996). In contrast, carnivorous fishes such as mummichogs may be able to assimilate more than the soluble fraction (CH$_3$Hg-TAM) in prey. Dietary CH$_3$Hg bioavailability appears to be considerably influenced by digestive processes of consumers in addition to the physicochemical form of CH$_3$Hg in the diet.

Effect of digestive processes on metal bioavailability to predators

There have been relatively few studies that have specifically investigated the role of digestive processes in dietary metal bioavailability to fish (e.g., Fair and Sick 1984; Farag et al. 2000; Leaner and Mason 2002). Dietary metal assimilation in predators requires the digestion of prey tissues and solubilization of organically bound metals, which subsequently allow biologically incorporated metals in prey to become available to predators (Campbell et al. 2005). An in vitro solubilization technique using gut fluid extracted from deposit-feeding invertebrates has been applied in many studies and has been proven to be a reliable estimator of metal bioavailability from sediments (Mayer et al. 1996, 2001; Lawrence et al. 1999). In the present study, a substantial amount of Cd (68%–94% of the whole body burden) was released from benthic invertebrates and juvenile fish by mummichog gut fluid, which is considerably higher than Cd solubilized from sediments by marine invertebrates gut fluid (< ~20%) (Weston and Maruya 2002; Weston et al. 2004). A study conducted by Farag et al. (2000) using an in vivo digestion approach has shown that cutthroat trout (*Oncorhynchus clarkii*) solubilized 40%–60% of Cd whole body burden in field-collected benthic invertebrates. Despite high solubility of Cd in the digestive tract, however, only a small fraction of ingested dietary Cd is assimilated by fish (in the present study, 2%–11% by mummichogs). Low Cd assimilation in the digestive tract in fish may be due to extremely slow permeation of solubilized Cd through the surface (mucus) layer of gut membrane (Chowdhury et al. 2004). Unlike some invertebrate species, which may reabsorb Cd bound to the digestive gland, noninternalized Cd in the mucus layer in fish is eventually eliminated by sloughing (Handy 1996; Chowdhury et al. 2004). This elimination process may be considered as a defense mechanism against toxic trace elements such as Cd and suggests that fish may be able to efficiently regulate dietary Cd assimilation in the digestive tract (Chowdhury et al. 2004), regardless of its intracellular distribution in prey or solubility in digestive fluid.

The proportion of CH_3Hg solubilized (CH_3Hg-GSM) from a variety of benthic invertebrates and juvenile fish prey species by mummichog gut fluid in the current study also failed to show a general relationship with CH_3Hg AEs (CH_3Hg-AE). However, species-specific CH_3Hg-GSM% was generally comparable with CH_3Hg-AE% in most prey species. Similar results were also observed in a study by Leaner and Mason (2002), which demonstrated that CH_3Hg extracted from polychaetes (*Glycera americana*) with gastrointestinal fluids of channel catfish (*Ictalcurus punctatus*) was comparable with that assimilated by the catfish. In general, unlike inorganic metals, CH_3Hg is known to rapidly penetrate the gut membrane of fish and subsequently be assimilated and distributed among the tissues (Rouleau et al. 1998, 1999; Oliveira Ribeiro et al. 1999). Farmanfarmaian and Socci (1984) have demonstrated the rapid penetration of CH_3Hg through an epithelial layer of the gut membrane of mummichogs, using an in vitro infusion approach. Furthermore, unlike its inorganic form, CH_3Hg is less efficient in inducing mucus secretion and also much less likely to be trapped in the mucus layer (Farmanfarmaian 1985). All these findings suggest that digestive solubilization of CH_3Hg may substantially contribute to its assimilation efficiency in fish.

Potential mechanisms of trophic transfer of Cd and CH_3Hg

Despite an increasing awareness of the importance of dietary metal transfer, the role of predator–prey coupling in trace metal cycling in marine ecosystems is still not fully understood. Previous studies have shown a potential effect of intracellular metal storage in dietary sources on metal trophic transfer to consumers (e.g., Wallace and Lopez 1997; Nott 1998; Cheung and Wang 2005). Although some studies have demonstrated a direct 1:1 relationship between metal storage in the diet and metal assimilation in consumers (Reinfelder and Fisher 1991; Reinfelder and Fisher 1994; Wallace and Lopez 1996), others have often failed to find any consistent relationship (e.g., Rainbow et al. 2006; Zhang and Wang 2006; Steen Redeker et al. 2007). Except for some invertebrates with simple digestive physiology (e.g., copepods and bivalve larvae) (Reinfelder and Fisher 1991; Hutchins et al. 1995; Mason et al. 1995), it may be oversimplifying to seek a direct relationship between metal storage in prey and metal assimilation in predators without incorporating other physiological and biochemical processes (Wallace et al. 2008). Based on the current study, the TAM compartment should be redefined as fractions that are only available to (but not necessarily assimilated by) predators. Each predator's particular digestive physiology is likely to determine whether the available fractions in prey are equivalent to the assimilated fractions by predator (Wallace et al. 2008).

The results from the current study have demonstrated the importance of intracellular metal storage in prey as part of the metal trophic transfer processes. A significant relationship (approximately 1:1) between metal storage in a variety of prey species and metal solubilization in the digestive tract of mummichogs observed in this study shows that intracellular partitioning of metals in prey does contribute to metal trophic availability in the gut environment. However, a variety of physiological and biochemical processes in the gut environment of the predator may complicate and obscure the direct relationship between metal intracellular storage in prey and actual assimilation into the predator's tissues (Wallace et al. 2008).

Moreover, the present study also provided evidence for a possible mechanism previously suggested for a substantial difference in AE between inorganic (Cd) and organic (CH_3Hg) metals. Specifically, the results showed that intracellular storage of both Cd and CH_3Hg in prey organisms might affect solubilization in the digestive tract of a predator. The solubility of Cd in the digestive tract of a predator is, however, unlikely to directly influence its AE, which is probably limited by its gut membrane transport (Handy 1996; Chowdhury et al. 2004). On the other hand, the AE of CH_3Hg is likely to be influenced by its solubility in the digestive tract of a predator, rather than its gut membrane transport (Leaner and Mason 2002). Thus, the current study suggests that Cd trophic transfer is predator-dependent, while CH_3Hg trophic transfer is a prey-dependent process. Nevertheless, further studies are still required to establish its general applicability to other prey–predator couplings in aquatic ecosystems.

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Estimation of seal pup production from aerial surveys using generalized additive models

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Abstract: In this paper, we estimate the pup production of harp seals (*Pagophilus groenlandicus*) using generalized additive models (GAMs) based on thin-plate regression splines. The spatial distribution of seal pups in a patch is modelled using GAMs, and the pup production is estimated by numerically integrating the model over a fine grid area of the patch. Closed form expression for estimation of the the standard error of the pup production estimate is derived. The estimators are applied to simulated seal populations to investigate their properties. The results show that the proposed pup production estimator is comparable with the conventional pup production estimator. However, the bias of the standard error estimator of the proposed method is much lower than the bias of the conventional standard error estimator. The decrease of standard error bias results in a considerable reduction of the coefficient of variation estimate using the proposed GAM-based method. The proposed method is also applied to real survey data of harp seals obtained from aerial surveys in the Greenland Sea pack ice in 2002. We show that the number of pups counted from aerial photographs possess a good fit to the negative binomial distribution when a logarithmic link function is applied. The approach described here is applicable to many situations where georeferenced counts or measurements are available.

Résumé : Nous estimons, dans ce travail, la production de petits chez les phoques du Groenland (*Pagophilus groenlandicus*) à l'aide de modèles additifs généralisés (GAMs) basés sur des splines de régression en plaque mince. Nous modélisons la répartition spatiale des jeunes phoques dans une parcelle à l'aide de GAMs et nous estimons la production de jeunes en intégrant le modèle numériquement sur une surface en grille fine de la parcelle. Nous dérivons une expression en forme fermée pour estimer l'erreur type de l'estimation de la production de jeunes. Les estimateurs sont appliqués à des populations simulées de phoques afin d'en étudier les propriétés. Les résultats montrent que l'estimateur proposé de production des jeunes se compare à l'estimateur courant de production de petits. Cependant, le biais de l'estimateur de l'erreur type de la méthode proposée est beaucoup plus faible que celui de l'estimateur courant de l'erreur type. La réduction du biais de l'erreur type a pour résultat une réduction considérable de l'estimation du coefficient de variation en utilisant la méthode proposée basée sur les GAM. Nous avons aussi utilisé la méthode proposée avec de vraies données d'inventaire de phoques du Groenland obtenues par relevés aériens sur la banquise de la mer du Groenland en 2002. Nous démontrons que le nombre de petits dénombrés sur les photographies aériennes s'ajuste bien à la distribution binomiale négative lorsqu'on utilise une fonction log-link. La méthodologie que nous décrivons s'applique à de nombreuses situations dans lesquelles des dénombrements ou des mesures géoréférencés sont disponibles.

[Traduit par la Rédaction]

Introduction

Scientific advice for the management of exploited resources is based on biological assessments. The ice breeding harp (*Pagophilus groenlandicus*) and hooded (*Cystophora cristata*) seals are both abundant species in the North Atlantic, where they are the focus of highly controversial hunts conducted by Canada, Greenland, Norway, and Russia. The two species occur in two (hooded seals) or three (harp seals) putative populations that have historically been exploited and managed separately (ICES 2006*a*, 2006*b*). Thus, there is considerable interest in assessing the status and monitoring changes in abundance in all populations to manage the respective harvests responsibly. In addition, knowledge of seal population sizes are one factor required to estimate the potential influence of these species on other marine organisms, including commercially important fish species (e.g., Hammill and Stenson 2000; Nilssen et al. 2000; Bundy 2001).

Owing to uncertainties in the assumptions required when estimating abundance from catch-at-age data, sequential population models, and mark–recapture data, independent estimates of pup production, using photographic or visual aerial strip transect surveys, have been developed to determine population size of harp and hooded seals in the northwest Atlantic (Bowen et al. 1987; Hammill et al. 1992; Stenson et al. 2003), the Greenland Sea (Øritsland and Øien 1995; Haug et al. 2006; Salberg et al. 2008), and in the

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White Sea (Potelov et al. 2003; ICES 2006*b*). The total abundance is subsequently estimated by fitting a population model to the independent estimates of pup production while incorporating removals and reproductive rates (e.g., Healey and Stenson 2000; Hammill and Stenson 2007; Skaug et al. 2007).

To estimate pup production using aerial strip transects (see Kingsley et al. 1985; Stenson et al. 1993), the number of seal pups are counted either visually along an entire transect (with a known strip width) or on photographs taken along the transect. Each transect covers the entire area where seals occur. A number of parallel transects are surveyed to cover the entire patch. This survey design (hereafter referred to as the conventional method) estimates the total number of pups in the patch by multiplying the counted number of pups by a factor equal to the ratio between the total patch area and the sampled area.

The uncertainty in these pup production estimates are estimated based upon serial differences between the total number of pups counted on adjacent transects (Kingsley et al. 1985; Stenson et al. 1993). This assumes equal mean values between two neighboring transects. For seal pup count data, this assumption may not be valid because of clustering of whelping females. If the degree of clustering is high, we will have an unwanted contribution from the difference between the transect count mean values. This contribution will lead to an overestimate of the variance, and this bias will increase as the distance between transects increases. Alternatively, the variance may be underestimated if there is major variation in density along a transect that is not reflected in the transect totals.

In this paper, we propose a method for estimating the seal pup production using generalized additive models (GAMs; Hastie and Tibshirani 1990; Borchers et al. 1997; Wood 2006*a*) to model the seal pup density (or seal pup counts) in a patch as a function of spatial position. By assuming that the number of pups counted from aerial photographs are distributed as negative binomials and using thin-plate regression splines, the GAM provides us with an estimate of the expected seal pup density at each spatial location in the patch. Numerical integration of the density estimates will provide an estimate of total pup abundance.

One of the major challenges of using GAMs for abundance estimation has been to develop a simple method to estimate the standard error, or survey precision (Petitgas 2001), and resampling or simulation has often been required for that purpose (Borchers et al. 1997; Augustin et al. 1998; Wood 2006*b*). In this study, we propose a simple, closed form expression of the error variance of the total number of pups observed in an aerial survey. Unlike the existing computationally intensive methods, the proposed method gives an estimate of the error variance with low computational complexity. Moreover, contrary to the standard error estimates applied in Kingsley et al. (1985) and Stenson et al. (1993), the GAM-based standard error estimate does not rely on constructions of transects. For the GAM method, the standard error estimate is based on pup density differences between neighboring sampling points, instead of differences between transects counts.

To determine the characteristics of the method, it is first applied to simulated data sets and compared with the previously used method. We then apply the new method to data obtained during a photographic survey of harp seal pups in the Greenland Sea during 2002 (Haug et al. 2006).

Materials and methods

The Greenland Sea 2002 surveys

Aerial surveys were carried out in 2002 in the Greenland Sea pack ice to assess the pup production of the Greenland Sea population of harp seals (see Haug et al. 2006 for survey details). Reconnaissance surveys of areas historically used by harp seals in the Greenland Sea revealed three breeding patches that were subsequently surveyed photographically and (or) visually (Fig. 1). Each photograph was georeferenced, and the number of pups on each photograph was determined by experienced readers. In this study, we use photographs from two of the patches (B and C, see Haug et al. 2006) to compare estimates obtained using the conventional and GAM-based methods.

Conventional survey analysis

Surveys are based on a systematic sampling design with a single random start and a sampling unit of a transect of variable length. The number of pups on the *j*th transect is estimated as (Stenson et al. 1993)

$$t_j = \frac{\ell_j}{\sum_{k=1}^{P_j} f_k} \sum_{k=1}^{P_j} n_{k,j} \qquad (1)$$

where ℓ_j is the length of the transect, P_j is the number of photographs taken along the transect, f_k is the photograph frame length of the *k*th photograph, and $n_{k,j}$ is the number of pups counted in the *k*th photograph of the *j*th transect. Let T_i denote the transect spacing (in metres, m) and W_i denote the transect width (m). The transect width is estimated as the average of all photograph frame widths f_k in the patch. The estimated number of pups for the *i*th survey is given by

$$N_i^{\text{conv}} = v_i \sum_{j=1}^{J_i} t_j \qquad (2)$$

where J_i is the number of transects in the *i*th survey, and $v_i = T_i/W_i$ is the extrapolation factor for the *i*th survey determined by dividing the transect interval by the transect width.

For the conventional method, estimates of error variance are based on the serial differences between transects (e.g., Stenson et al. 1993) and assumes that the number of pups on the *j*th transect is modelled as

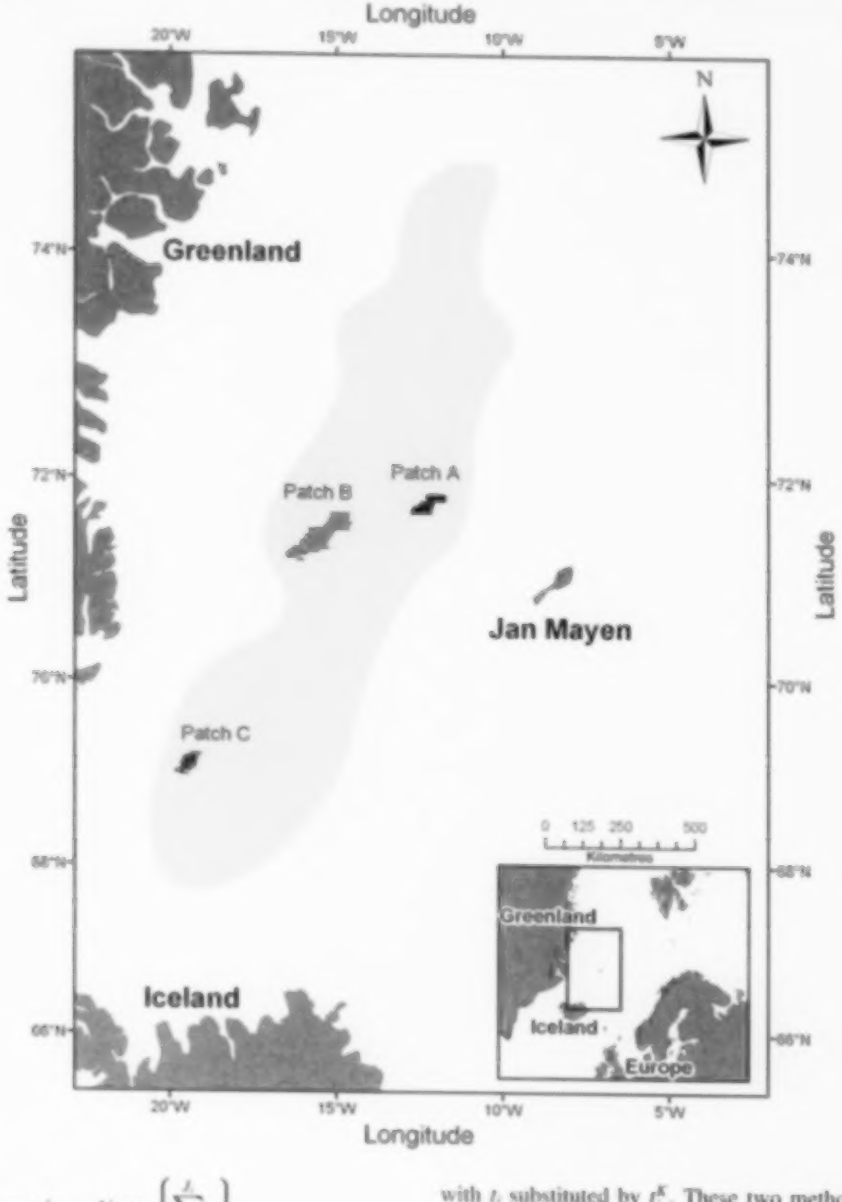
$$t_j = m_j + \varepsilon_j \qquad (3)$$

where m_j is the mean value, and ε_j is a random component. Now, assuming that two consecutive transect counts have equal mean values, i.e., $m_j = m_{j+1}$, and that ε_j and ε_{j+1} are statistically independent and with equal variance, we have

$$\text{var}\{t_j\} = \text{var}\{\varepsilon_j\} = (1/2)\ \text{var}\{t_j - t_{j+1}\} \qquad (4)$$

Hence, the error variance may be expressed as (Stenson et al. 1993; Haug et al. 2006)

Fig. 1. Survey area in the Greenland Sea with three seal patches (A, B, and C); shaded area indicates where fixed-wing reconnaissance surveys where flown.


Longitude
20°W
15°W
10°W
5°W
N
74°N
72°N
70°N
68°N
66°N
Latitude
Greenland
Patch A
Patch B
Patch C
Jan Mayen
Iceland
0 125 250 500
Kilometres
Greenland
Iceland
Europe

$$
\text{(5)}\qquad \operatorname{var}\{N_i^{\text{conv}}\} = v_i(v_i-1)\operatorname{var}\left\{\sum_{j=1}^{J_i} t_j\right\} = v_i(v_i-1)\frac{J_i}{2(J_i-1)}\sum_{j=1}^{J_i-1}(t_j-t_{j+1})^2
$$

In the method proposed in Kingsley and Smith (1981) and Kingsley et al. (1985), the transect counts t_i are modified according to $t_i^K = t_i - RA_i$, where R is an estimate of the average pup density of the whole patch, and A_i is the area of the ith transect. The variance is then estimated using eq. 5, but with t_i substituted by t_i^K. These two methods are asymptotically the same. If the transect length is equal for all i, then they are equal. In a seal survey, the length of the transects are confined by the extent of the seal patch. Thus, continuing sampling outside the patch to ensure equal transect lengths would not increase the counts. However, the difference between the methods is minor even if the transect length changes.

For the seal pup count data, the assumption of equal mean counts, or modified counts, between two adjacent transects is most likely not valid (especially if v_i is large and the degree of clustering is high). Hence, we will have an unwanted contribution from the difference between the transect count mean values, and this can lead to an overestimate of the variance of the pup production estimate.

GAM survey analysis

In the proposed survey analysis, we model the expected seal density over the whole patch. The data are analyzed using the spatial modelling methods based on GAMs. The counted number of pups in the *i*th photograph is

$$(6)\qquad n_i = A_i d_i$$

where A_i is the area covered by the *i*th photograph, and d_i is the density of pups in photograph *i*. We assume that n_i is negative binomial distributed with a mean value μ_i and shape parameter κ. Even thought we are dealing with count data, a Poisson error distribution would not be appropriate, since the seal count data are overdispersed. Overdispersed data are often a consequence of the population being clustered (McCullagh and Nelder 1989). We therefore assume that the data are negative binomial distributed, which has been applied previously to model overdispersed count data (Gardner et al. 1995; Hinde and Demétrio 1998; Thurston et al. 2000).

The purpose of the GAM is to model the pup density over the patch as a function of spatial location $\mathbf{x} = [x_1, x_2]^T$. The photographs are treated as independent samples by definition of the GAM framework (Green and Silverman 1994; Petitgas 2001), and hence all spatial structure is modelled by the trend surface defined below. Using the logarithmic link function $g(\mu_i) \triangleq \eta_i = \log(\mu_i)$, where $\mu_i = E[n_i]$, the pup density is modelled as

$$(7)\qquad \mu_i = e^{\eta_i} = \exp[\log(A_i) + S(\mathbf{x}_i)]$$

where $\log(A_i)$ is an offset variable, and $S(\cdot)$ is a smoothing function of the spatial covariables, and $\mathbf{x}_i$ is the spatial location at the *i*th sampling point. Note that the estimated expected seal density is then

$$(8)\qquad E\{d_i\} = \exp[S(\mathbf{x}_i)]$$

As in Wood (2003), we assume that the smoothing function is a thin-plate smoothing regression spline. The smoothed surface $S(\mathbf{x})$ at spatial location $\mathbf{x} = [x_1, x_2]^T$ may be expressed as (Green and Silverman 1994; Wood 2003)

$$(9)\qquad S(\mathbf{x}) = \sum_{i=1}^{N_{\text{photo}}} \delta_i \eta(\| \mathbf{x} - \mathbf{x}_i \|) + \sum_{j=1}^{3} \alpha_j \phi_j(\mathbf{x})$$

where $\eta(r) = (1/16\pi)r^2 \log(r^2)$, $\phi_1(\mathbf{x}) = 1$, $\phi_2(\mathbf{x}) = x_1$, $\phi_3(\mathbf{x}) = x_2$, and N_{photo} is the number of photographs in the patch. The parameters δ_i and α_j are estimated using the algorithms given in Wood (2003). One of the key points in Wood (2003) is reduced rank modelling of the estimation problem, in the sense that the smoothers are constructed by an eigen decomposition and truncation of the solution of the thin-plate spline smoothing problem. The obtained basis is optimal in the sense that the truncation is designed to result in the minimum possible perturbation of the thin-plate spline smoothing problem for a given basis dimension. The maximum number of degrees of freedom refers to the dimension of the truncated basis. Hence, the N_{photo} variables δ_i, $i = 1, \ldots, N_{\text{photo}}$ may be modelled using only M variables, and the total number of unknowns to estimate is $M + 3$ given by $\boldsymbol{\beta} = [\beta_1, \beta_2, \ldots, \beta_M, \alpha_1, \alpha_2, \alpha_3]^T$.

Under the assumption of exponential family data, the GAM may be fitted to the data using iterated reweighted least squares (Hastie and Tibshirani 1990). However, since the negative binomial distribution is only included in the exponential family for known shape parameter κ, the algorithm may only be used to fit the GAM for fixed κ. To estimate the μ_i values and the shape parameter κ in the GAM model, we alternate between holding κ fixed and updating the μ_i values using the method suggested in Wood (2003) and holding the μ_i values fixed and updating κ according to the method described in Thurston et al. (2000) and Svetliza and Paula (2001).

A smoothing parameter λ plays an important role in the GAM framework using thin-plate regression splines (Wood 2003). Since the underlying model assumption of a GAM is that the spatial information is in the trend surface, the smoothing parameter should be designed such that the estimated trend surface captures the variability of spatial trend, but not the random noise. The smoothing parameter λ is estimated as the value that minimizes the generalized cross validation (see e.g., Green and Silverman 1994; Wood 2006*a*).

If the GAM contains more than one term (we have only the term $S(\mathbf{x})$ in our model), the analysis may be extended following the lines of Wood (2003, 2006*a*, 2006*b*).

Abundance estimation

Once the model has been chosen, we may use eq. 9 to predict the seal density at any location in the patch. Hence, the GAM provides us with a smoothed expected seal density surface over the entire survey area. To estimate the total pup production in the patch, we numerically integrate the expected seal density surface over space (Borchers et al. 1997; Augustin et al. 1998):

$$(10)\qquad N_{\text{GAM}} = \sum_{\text{fine grid}} N_{\text{GAM}}^{j} = \sum_{\text{fine grid}} \exp[S(\mathbf{x}_j)]A_j^{\text{g}}$$

where N_{GAM}^{j} is the estimated number of seals in the *j*th fine grid area at spatial location $\mathbf{x}_j$, and A_j^{g} is the corresponding fine grid area.

Variance and bias estimation

Below we list three different methods for estimating the variance and bias of the pup production estimator. Method I is developed in this paper, and it is a simple closed form expression for the variance of the pup production estimate that easily can be computed. For completeness, we include other previously published methods for estimating the survey precision. These methods have been denoted Method II and Method III. Method II was applied in analysis of fish egg production in (Borchers et al. 1997; Augustin et al. 1998), and Method III was recently applied to estimate confidence intervals for nonlinear function of the free parameters $\boldsymbol{\beta}$ (Wood 2006*b*). Method II and Method III are robust and precise methods, but they are computational intensive.

Let $\mathbf{X}_g$ denote the matrix that linearly maps the parameter vector to fine grid estimates $\mathbf{s}_g$ of the smoothed curve, i.e., $\mathbf{s}_g = \mathbf{X}_g\boldsymbol{\beta}$. The covariance matrix of $\mathbf{s}_g$ for a given smoothing parameter λ is then

$$(11)\qquad \mathrm{cov}\{\mathbf{s}_g\} = \mathbf{X}_g\,\mathrm{cov}\{\boldsymbol{\beta}\}\mathbf{X}_g^T$$

where $\mathrm{cov}\{\boldsymbol{\beta}\}$ is the covariance matrix of the estimated parameter vector $\boldsymbol{\beta}$.

Method I

To estimate the variance of N_{GAM}, we apply the delta method. A first-order approximation of the variance of N_{GAM} may be written as

$$(12)\qquad \mathrm{var}\{N_{\mathrm{GAM}}\} = \frac{\partial N_{\mathrm{GAM}}}{\partial \mathbf{s}_g^T}\,\mathrm{cov}\{\mathbf{s}_g\}\,\frac{\partial N_{\mathrm{GAM}}}{\partial \mathbf{s}_g} = \frac{\partial N_{\mathrm{GAM}}}{\partial \boldsymbol{\beta}^T}\,\mathrm{cov}\{\boldsymbol{\beta}\}\,\frac{\partial N_{\mathrm{GAM}}}{\partial \boldsymbol{\beta}}$$

For the logarithmic link function we have

$$(13)\qquad \frac{\partial N_{\mathrm{GAM}}}{\partial S(\mathbf{x}_j)} = A_j^g \exp[S(\mathbf{x}_j)] = N_{\mathrm{GAM}}^j$$

and hence we have the compact form of the variance of the pup production estimate

$$(14)\qquad \mathrm{var}\{N_{\mathrm{GAM}}\} = \boldsymbol{\mu}_g^T\,\mathrm{cov}\{\mathbf{s}_g\}\boldsymbol{\mu}_g = \boldsymbol{\mu}_g^T\mathbf{X}_g\,\mathrm{cov}\{\boldsymbol{\beta}\}\mathbf{X}_g^T\boldsymbol{\mu}_g$$

A Bayesian-type estimate of the covariance matrix of $\boldsymbol{\beta}$ provided by Silverman (1985) was applied to calculate eq. 14.

The bias may also be estimated using the delta method. However, if we apply a first-order approximation of N_{GAM}, the bias is equal to zero. If we use a second-order approximation of N_{GAM}, the bias may be expressed as

$$(15)\qquad \mathrm{bias}\{N_{\mathrm{GAM}}\} = \frac{1}{2}\mathrm{trace}[\mathrm{diag}(\boldsymbol{\mu}_g)\,\mathrm{cov}\{\mathbf{s}_g\}] = \frac{1}{2}\mathrm{trace}[\mathrm{diag}(\boldsymbol{\mu}_g)\mathbf{X}_g\,\mathrm{cov}\{\boldsymbol{\beta}\}\mathbf{X}_g^T]$$

Method II

Another method for estimating the precision of the pup production estimate is the simulation technique proposed in Borchers et al. (1997) and Augustin et al. (1998). This method utilizes the assumption that the number of seals in a given photograph follows a negative binomial distribution parameterized by the estimated parameters μ_i and κ.

First, we sample B replicas in the sampling points

$$(16)\qquad n_i^b = \mathrm{neg.bin}(\mu_i, \kappa), \qquad i = 1, \ldots, N_{\mathrm{photo}}, \quad b = 1, \ldots, B$$

where μ_i and κ are the estimated mean value and shape parameter, respectively. Then, we run the GAM scheme to estimate the pup density in each fine grid location and estimate the number of pups in the patch

$$(17)\qquad N_{\mathrm{GAM}}^b = \sum_{\text{fine grid}} \exp[S^b(\mathbf{x}_j)]A_j^g, \qquad b = 1, \ldots, B$$

Based on the estimated number of pups from the simulations, we may express the variance of N_{GAM} as

$$(18)\qquad \mathrm{var}\{N_{\mathrm{GAM}}\} = \frac{1}{B-1}\sum_{b=1}^{B}(N_{\mathrm{GAM}}^b - \overline{N}_{\mathrm{GAM}})^2$$

where $\overline{N}_{\mathrm{GAM}}$ is the empirical mean value of all the N_{GAM}^b values. Using the bootstrap procedure, we also obtain an estimator for the bias of the pup production estimate

$$(19)\qquad \mathrm{bias}\{N_{\mathrm{GAM}}\} = \overline{N}_{\mathrm{GAM}} - N_{\mathrm{GAM}}$$

Note that this method, contrary to Method I, incorporates smoothing parameter and shape parameter uncertainties into the variance estimate.

Method III

Instead of applying the delta method to find an approximate expression of the variance, it may be obtained by simulations from the posterior distribution of $\boldsymbol{\beta}$ (Silverman 1985; Wood 2006*b*)

$$(20)\qquad \boldsymbol{\beta}^b \sim \mathcal{N}(\boldsymbol{\beta}, \mathrm{cov}\{\boldsymbol{\beta}\})$$

where $\mathrm{cov}\{\boldsymbol{\beta}\}$ also here is a Bayesian-type estimate of the covariance matrix of $\boldsymbol{\beta}$ (Silverman 1985).

From each of the B Monte Carlo runs, fine grid mean value estimates $\mu_j^b = A_j^g \exp[S^b(\mathbf{x}_j)]$, $b = 1, 2, \ldots, B$, are calculated. Then, the pup production are estimated as in eq. 17, and the variance and bias are calculated as in eqs. 18 and 19, respectively.

Diagnostics

Simulated survey data

The performance of the proposed estimators was evaluated using Monte Carlo simulations on simulated surveys with different degrees of clustering and habitat structure. For each Monte Carlo run, a simulated whelping patch was generated where the positions of the seal pups were randomly distributed.

Gaussian distributed clusters

In this scenario, the positions of the seal pups were randomly distributed according to a three-component, bivariate, Gaussian mixture distribution. The parameters used for the various components were

$$(21)\qquad \boldsymbol{\mu}_1 = [1, 4]^T, \quad \boldsymbol{\mu}_2 = [-3, 0]^T, \quad \boldsymbol{\mu}_3 = [-4, -5]^T$$

and

$$(22)\qquad \boldsymbol{\Sigma}_1 = \begin{bmatrix} 2 & 0 \\ 0 & 1/2 \end{bmatrix}, \quad \boldsymbol{\Sigma}_2 = \begin{bmatrix} 1 & 2/3 \\ 2/3 & 3 \end{bmatrix}, \quad \boldsymbol{\Sigma}_3 = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}$$

The mixture components were $p_1 = p_2 = p_3 = 1/3$. Here the superscript T denotes the vector transpose. For each component, we generated 2000 seals, making a total of 6000 seals. The simulated patch was artificially photographed, and the number of seals in each photograph was then counted and served as input data for the estimator.

Non-Gaussian distributed clusters

In this scenario the positions of the seals were randomly distributed according to four homogeneous Poisson processes (Martinez and Martinez 2002). The intensities used for the various Poisson clusters were as follows: $\lambda_1 = 5$,

$\lambda_2 = 3$, $\lambda_3 = 0.5$, and $\lambda_4 = 2$, with the radiuses $r_1 = 10$, $r_2 = 10$, $r_3 = 20$, and $r_4 = 15$. The following translation vectors were used for the various Poisson clusters:

$$(23)\qquad \begin{array}{ll} \boldsymbol{\mu}_1 = [0, 25]^T, & \boldsymbol{\mu}_2 = [0, -25]^T, \\ \boldsymbol{\mu}_3 = [0, -25]^T, & \boldsymbol{\mu}_4 = [0, 0] \end{array}$$

In total, 3000 seals were generated, and the simulated patch was artificially photographed.

Uniformly distributed clusters

In this scenario, the positions of the seal pups were randomly distributed according to a bivariate uniform distribution.

Goodness of fit

Since both the GAM-based abundance estimate and the corresponding variance estimate are model dependent, the goodness of fit of the statistical model need to investigated. Ideally the residuals should be independent and contain no unmodelled spatial correlations. Also, the residual variance should be homogeneous over the patch. If there are substantially unmodelled spatial correlation in the residuals, this may yield negatively biased estimates of variance (Borchers et al. 1997).

The deviance residuals $D(y, \mu, \kappa)$ were investigated to evaluate goodness of fit of the negative binomial distribution. As in Pierce and Schafer (1986), we use the adjusted deviance

$$(24)\qquad D_A(y, \mu, \kappa) = D(y, \mu, \kappa) + \rho_3(\mu, \kappa)/6$$

where $\rho_3(\mu, \kappa) = (\kappa + 2\mu)/\sqrt{\kappa\mu(\kappa + \mu)}$ is the standardized skewness of the negative binomial distribution. It has been reported that the fit of the adjusted deviance residuals is close to the normal distribution (Pierce and Schafer 1986). However, χ^2 tests are usually unreliable, since the deviance is constructed from a model that has as many parameters as data (Wood 2006*a*, p. 70).

We investigated the distribution and homogeneity of variance of the deviance using a variance-stabilized percent–percent plot (pp-plot) (Michael 1983) and by studying the residuals as a function of predicted values. Comparisons between the goodness of fit of the negative binomial and Poisson distributions were performed, which indicated that the negative binomial distribution is a better candidate than the Poisson distribution. A Poisson distribution with an overdispersed structure was also applied, but did not provide as good fit to the data as the negative binomial distribution.

The spatial structure of the residuals was evaluated using several approaches. First, we visually inspected the deviance surface a function of spatial position (covariates), and a Mantel test (see e.g., Manly 1997; Granadeiro et al. 2004) was then applied to test whether any spatial structure was present in the deviance residuals. Two symmetric distance matrices whose rows and columns correspond to the same set photographs were calculated. One of the distance matrices was based on the absolute difference in deviance between all pairs of photographs, and the other distance matrix was based on the Euclidean distances between photographs. The Mantel correlation (Manly 1997) was first calculated for the correlation between the original matrices and used as a reference value for the test. The rows and columns of the matrix corresponding to one of the distance matrices were permuted 2500 times, and the Mantel correlation was recalculated for each permutation. The residuals were considered to be spatially unstructured if the Mantel correlation between the original matrices was within the 2.5% and 97.5% quantiles of the distribution of the Mantel statistic based on randomization (Manly 1997; Granadeiro et al. 2004).

In addition, as an alternative to deviance residuals, the goodness of fit was investigated by inspecting the randomized quantile residuals developed in Dunn and Smyth (1996).

Results

In the GAM-based estimations, the maximum number of degrees of freedom was selected to be 30 for the simulated data based on Poisson clusters, 50 for the simulated data based on Gaussian clusters, and 50 for the real data case.

Simulated survey data

For all examples, 1000 Monte Carlo simulations were run. The total number of seals present was estimated using the GAM-based method and the conventional method. Realizations of the synthetic patches are shown (Figs. 2*a*, 2*c*). The dots represent randomly distributed seals, and the squares represent the locations where the artificial photographs were taken. The overall photographic coverage obtained along a transect line was about 80% in both examples. The estimated seal patch densities for the various simulated patches are shown (Figs. 2*b*, 2*d*).

Gaussian distributed clusters

The main proportion of synthetic seals were distributed in three clear clusters (Figs. 2*a*, 2*b*). The empirical distribution of the GAM-based estimator and the conventional method are also shown (Figs. 3*a*, 3*b*). The vertical broken line indicates the true value. We see that both methods have a clear peak around 6000 seals. The mean value of the Monte Carlo simulations for the GAM method is $\mu_{\mathrm{GAM}} = 5982$, and the mean value of the conventional method is $\mu_{\mathrm{conv}} = 5957$. From the figure, we see that both methods have approximately the same variance. Hence, both abundance estimators appeared to perform equally well in this example.

The empirical distributions of the estimated standard error of the pup production estimator for the GAM-based method and the conventional method are shown (Fig. 4*a*). For the GAM-based method, we used Method 1 to estimate the standard error of the pup production estimate. The estimates were calculated using eq. 14 for the GAM method and eq. 5 for the conventional method. The broken vertical line represents the true values of the standard error of the pup production estimates. For the GAM-based method (shown in the left histogram), we see that the standard error estimator slightly overestimates the estimation error. However, for the conventional method (shown in the right histogram), we see that the error estimates were much higher. We also see that the variance of the proposed survey precision estimator is less than the variance of the conventional survey precision estimator.

Fig. 2. Realizations and estimated densities of the synthetic patches. The dots represent randomly distributed seals. The squares represent the locations where the artificial photographs were taken. (*a*, *b*) Gaussian cluster; (*c*, *d*) Poisson cluster.

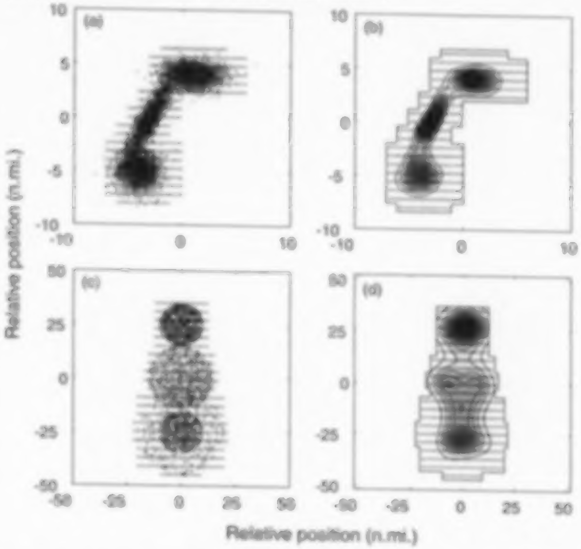


Fig. 3. Histogram for the estimated pup production for the simulated surveys: (*a*) Gaussian clusters and generalized additive model (GAM), (*b*) Gaussian clusters and conventional method, (*c*) Poisson clusters and GAM, and (*d*) Poisson clusters and conventional method. The broken vertical lines represent the true number of pups: $N = 6000$ (*a*, *b*) and $N = 3000$ (*c*, *d*).

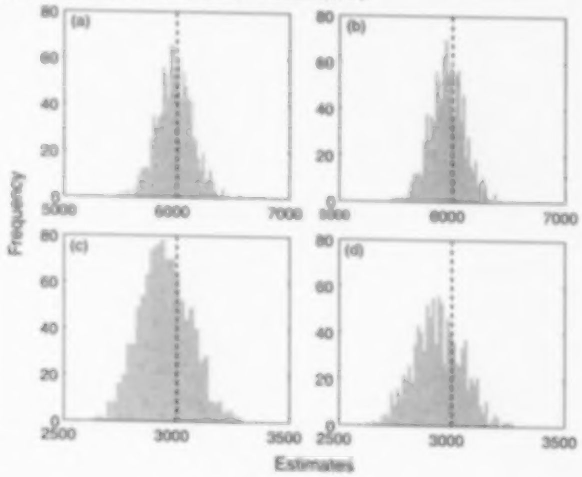
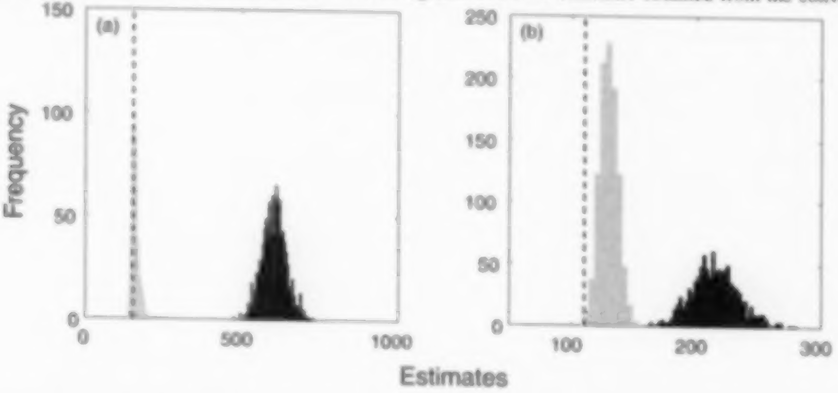


Fig. 4. Histogram for the estimated standard error of the pup production estimates for the simulated surveys: (*a*) Gaussian distributed clusters, (*b*) non-Gaussian distributed clusters. The broken vertical lines represent the true value of the standard error. Light shaded histograms show the estimates obtained from the GAM model; dark shaded histograms show the estimates obtained from the conventional method.



The same experiment was carried out for estimating the standard error of the pup production estimate using Method II and Method III. The simulations are not included because of space considerations. Methods I and III gave approximately equal results in terms of mean value and variance. Method II tended to overestimate the survey precision slightly more than Method I, but had the same variance as Methods I and III. However, the computational complexity with Methods II and III was much higher than with Method I. The main benefit is the dramatic cut in computational costs; where Methods II and III need hours to run, Method I provides precise estimates within seconds.

Non-Gaussian distributed clusters

The synthetic seals were distributed in four clusters with different densities (Fig. 2*c*). The empirical distribution of the GAM-based estimator and the conventional method are shown (Figs. 3*c*, 3*d*). In this example, we see that both methods tend to underestimate the pup production. The mean value of the Monte Carlo simulations for the GAM method is $\mu_{\mathrm{GAM}} = 2949$, and the mean value of the conventional method was $\mu_{\mathrm{conv}} = 2941$. We see that both abundance estimators slightly underestimates the pup production and seem to perform equally well.

The empirical distributions of the estimated standard error for the GAM-based method (left histogram) and the conventional method (right histogram) are shown (Fig. 4*b*). For the GAM-based method, we used Method I to estimate the standard error of the pup production estimate. We see that the GAM-based standard error estimator slightly overestimates the estimation error, whereas for the conventional method we see that the error estimates were much higher.

Uniformly distributed clusters

As expected, both methods for estimating the pup production and the variance of the pup production performed the same in this scenario. Figures showing the results for the uniformly distributed patch are not included.

Real aerial survey data

The GAM-based pup production estimation technique was now applied to estimate the seal pup abundance of the Greenland Sea in 2002.

Abundance estimates of Patch B and Patch C

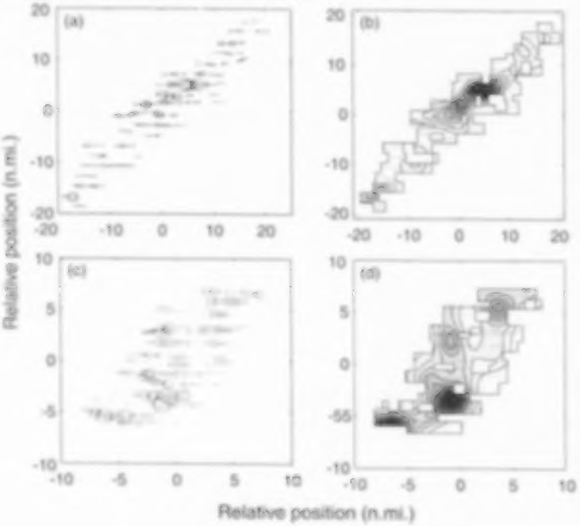
A survey of Patch B (70°52′N–71°25′N and 14°44′W–16°38′W) was successfully completed March 29 (Fig. 1). Twenty transects were flown in an east–west direction, spaced 2 nautical miles apart (1 nautical mile = 1.852 km). The relative sample locations of the seal density data of Patch B are shown (Fig. 5*a*). The symbol sizes are proportional to the observed pup density at the sampling location. A total of 5220 pups were counted on the 521 exposures obtained. Using the conventional survey analysis, the pup production was estimated to be 113 513 (SE = 24 933). The CV was estimated to be 22%. Using the GAM-based method, the pup production and standard error (using Method I) was estimated to be 114 184 (SE = 7569) (see also Table 1). Although the pup production estimates were very similar, the estimation error was considerably lower than for the conventional analysis, with the new CV being 6.63%. Using Methods II and III, the estimated survey precisions were SE = 7156 (CV = 6.52%) and SE = 7636 (CV = 6.44%), respectively. The estimated biases were less than 5% of the pup production estimate for all three methods (see Table 2).

The contour plot of the estimated pup density obtained using the GAM-based pup production estimator is also shown (Fig. 5*b*). The squares show the location of the photographs. In comparison with the sample density data (Fig. 5*a*), we see that the estimated density captures the spatial structure of the observed data.

The whelping Patch C was surveyed on April 6th. In total, 14 east–west transects spacing 1 nautical mile were flown covering the area between 69°01′N–69°14′N and 19°06′W–19°51′W (Fig. 5*c*). A total of 1282 pups were counted, and using the conventional survey analysis, pup production was estimated to 19 248 (SE = 2002, CV = 10.4%). Using the GAM-based method, the pup production and precision (using Method I) was estimated to be 21 280 (SE = 993; Table 1). The new CV was estimated to be 4.67%. For Methods II and III, the survey precisions were SE = 907 (CV = 4.31%) and SE = 1121 (CV = 5.06%), respectively. The biases were less than 5% for Patch C.

The contour plot for the estimated pup density obtained using the GAM-based pup production estimator is shown (Fig. 5*d*). When comparing the estimated pup density with the sample density (Fig. 5*c*), we see that the estimated density captures the structural behaviour of the observed data. All three GAM-based methods of estimating the survey precision give similar results in both surveys samples (Table 1).

Fig. 5. Relative sample locations (1 nautical mile (n.mi.) = 1.852 km) of seal density data for (*a*) Patch B and (*c*) Patch C: symbol sizes are proportional to the observed pup density at the sampling location. Contour plot of the expected seal density of (*b*) Patch B and (*d*) Patch C. The white empty areas in panels (*b*) and (*d*) are open water where the camera was turned off. The small squares represent the position of the photographs. Darker shading indicates higher seal density.



The survey precisions for patch B and C were also estimated using variance expression given by Fewster et al. (2008). For patch B and C, the precisions were estimated to be SE = 19016 (CV = 16.8%) and SE = 1980 (CV = 10.1%), respectively.

Goodness of fit

The adjusted deviance residuals and the randomized quantile residuals were investigated to evaluate the adequacy of the smoothing spline model. The adjusted deviance of the GAM model was 494 on 476 degrees of freedom (Patch B), whereas for Patch C the deviance was 342 on 285 degrees of freedom. The adjusted deviance residuals possessed a good fit to the normal distribution for Patches B and C (Figs. 6*a*, 6c). The GAM model was further investigated for any spatial structure in the adjusted deviance residuals (Figs. 7*a*, 7*b*). From the figures, we see that the deviance residuals may create small clusters, indicating some remaining unmodelled spatial correlations. However, the Mantel randomization test ($0.975 > p > 0.025$) did not indicate that there was any significant spatial structure remaining in the deviance residuals for Patch B, which also indicated the GAM fit to the data was good. The same Mantel test diagnostics evaluation of the deviance residuals was applied to the Patch C data. For Patch C, the output of the Mantel test was $p = 0.015$. However, the calculated Mantel correlation for Patch C was 0.04, which indicates that the effect of spatial correlation is fairly small. When the randomized quantile residuals were applied in the Mantel test instead of the deviance residuals, we obtained $p = 0.06$. Hence, no signifi-

Table 1. Pup production estimates and precision (SE, with percent coefficient of variation (CV %) in parentheses) as derived from analyses using the GAM method and conventional method for Patch B and Patch C in the Greenland Sea survey in 2002.

	Pup production estimate		Survey precision (CV %)			
	GAM	Conventional	Method I	Method II	Method III	Conventional
Patch B	114 187	113 513	7 569 (6.63%)	7 156 (6.52%)	7 636 (6.44%)	24 933 (22%)
Patch C	21 280	19 248	993 (4.67%)	907 (4.31%)	1 121 (5.06%)	2 002 (10.4%)

Note: Methods I–III are new methods to assess survey precision (see text for explanation).

Table 2. Degrees of freedom (df), shape parameter, and bias estimates as derived from analyses using the GAM method for Patch B and Patch C in the Greenland Sea survey in 2002.

			Bias for Methods I–III		
	df	Shape parameter	I	II	III
Patch B	44.5	0.88	5151	–4473	4374
Patch C	36.1	3.96	714	–240	882

Fig. 6. Percent–percent plot (pp-plot) between the adjusted deviance residuals and the zero-mean unity variance normal distribution of (*a*) Patch B and (*c*) Patch C. Deviance residual plots for the fitted negative-binomial-based GAM model for (*b*) Patch B and (*d*) Patch C.

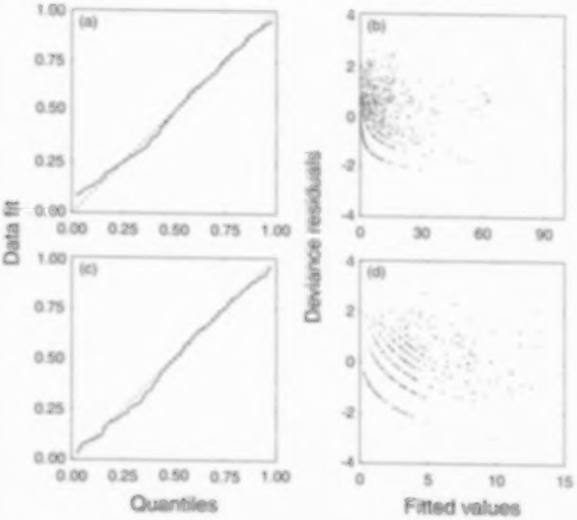
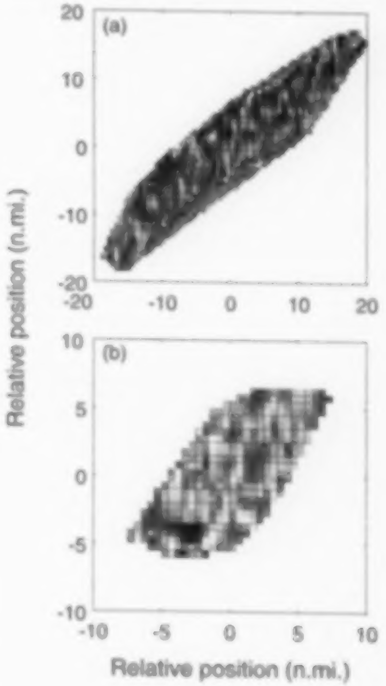


Fig. 7. Deviance residuals as a function of spatial location (1 nautical mile (n.mi.) = 1.852 km) of (*a*) Patch B and (*b*) Patch C.



cant remaining spatial structure was detected in the randomized quantile residuals.

The adjusted deviance residuals as a function of the fitted values are shown (Figs. 6*b*, 6*d*). The variance of the deviance residuals seems to be fairly constant over the range of fitted values; however, since there is a much higher concentration of data on the left side of the scale, it is hard to evaluate the homogeneity of the variance.

Discussion

In this paper, we have applied a GAM-based method with thin-plate regression splines for estimation of the seal pup production from aerial photographs. The number of seal pups counted from aerial photographs was assumed to be distributed as negative binomial with different mean values. Model diagnostics indicated that the negative binomial distribution yielded a good fit to the data. The Poisson distribution was also investigated as a possible candidate, but it was not able to capture the behaviour of the overdispersed data. By the use of thin-plate regression splines, the GAM model provided us with an estimate of the seal density at each spatial location in the patch. Abundance estimates were obtained by numerically integrating the estimated seal density provided by the GAM model. The proposed method is similar to those described in Augustin et al. (1998) and Hedley et al. (1999), but applies a 2D isotropic bivariate smoothing function of latitude and longitude, instead of sum of two 1D smoothing functions (one for latitude and one for longitude). Wood and Augustin (2002) remarked that "the assumption of having additive latitude and longitude effects may not be very realistic. A priori it is odd to model the dependence on longitude and latitude by summing a longitude and a latitude effect". Hence, the bivariate smoothing function is invariant of the orientation of the sampling transects. Along with the pup production estimate, the variance of the pup production estimate is an important quantity that was estimated. We derived a simple closed form estimator of the variance of the pup production estimate.

Previous estimation of harp seal pup production in the Greenland Sea (Haug et al. 2006), northwest Atlantic (Stenson et al. 2003), and White Sea (Potelov et al. 2003) have applied the conventional analysis approach. The results obtained using the proposed methods for estimating the pup production and estimating the survey precision were compared with the conventional methods. From the simulation-based analysis, we saw that both the conventional method and the proposed method for estimating the pup production provided very accurate estimates. Also, we saw that conventional method heavily overestimated the variance of the pup production estimate, whereas the estimated variance for the proposed method was close to the true value. The same simulations were carried out using uniformly distributed random seals instead of the clustered data. The conventional method and the proposed method provided very accurate results, both in terms of estimating the pup production and estimating the variance. In a scenario like this, the conventional method is expected to perform well, since the assumption of equal number of seals between transects was satisfied. Simulations indicate that when the degree of clustering among the data increases, the performance of the conventional method degrades, in the sense that the method tends to overestimate the variance of the pup production estimate. The proposed method for estimating the pup production appears to work well in both scenarios. Because of this, we are able to obtain more precise estimates of the CV quantity by using the GAM-based estimator. From the real data case study, the CV was reduced by a factor of two–three when comparing the proposed method with the conventional method. The numerical simulations have also been carried out on sparse data for both uniformly distributed seals and clustered seals, and similar results were seen.

For the real data, we obtained fairly close estimates of the pup production using the GAM-based method and the conventional method. The survey precision estimates obtained from the proposed GAM method were much lower than the estimates obtained from the conventional method. We can see that the real data has a clear clustered spatial structural behavior. Because of this, we can assume that the estimates of the survey precision obtained using the conventional method are highly overestimated. A minor source of error for the conventional method of estimating the survey precision is the assumption of parallel transects. Because of drift caused by strong winds, this can be a difficult task to achieve. From the photographs, we see that the transects are far from parallel and that the distance between the transects does indeed change. This is not an issue for the GAM method, since it relies on the density difference between neighboring sampling points rather than relying on the constructions of transects. This also makes the GAM method less sensitive to varying transect lengths and the degree of clustering of the data. The practical benefits of more precise estimates of the uncertainty of the pup production allow better monitoring of the population and quicker detection of changes in the population.

We have implemented Fewster's variance estimator (var_{O_1}), which was recommended for systematic transect when overlapping strata is possible (Fewster et al. 2008). The variance estimates obtained were approximately equal to the variance estimate obtained by the conventional method in both the simulated patches and the real data examples. The variance expression in Fewster et al. (2008) was derived for encounter rate variance estimation in distance sampling, and we needed to modify the variance estimator to fit the seal pup abundance estimation framework. Fewster's method is based on based on a random design, where the transect lengths are chosen randomly.

Comparisons with the resampling-based methods (Methods II and III) were carried out on the simulated survey data and the real survey data, but not included for the simulation-based survey because of space considerations. The simulations show that the proposed survey precision estimator was similar to the simulation-based estimators. The comparison for the real data found in Table 1 shows that all methods provided fairly close estimates.

The outcome of the Mantel test on Patch C was that the Mantel correlation obtained from the initial distance matrices was above the 97.5% quantile obtained from the randomization. However, the value of Mantel correlation was only 0.04, indicating that the spatial correlation is fairly small. Moreover, we also applied the Mantel test to the simulation studies (Gaussian clusters), and some spatial correlation was shown (in 90% of the Monte Carlo runs, a p value outside the 2.5%–97.5% quantiles was obtained). However, even though some spatial correlation in deviance residuals was shown, the variance was only slightly overestimated.

If the acquired photographs contain a large fraction of zero observations, problems with inference can occur when using GAM with standard negative binomial or Poisson error models (Barry and Welsh 2002). These problems occur because the negative binomial and Poisson assumption are not adequate approximations to the conditional distribution of the data. Moreover, if the photographs contain only a few seal pups, the estimates of the derivatives given in eq. 13 would be small, and the first-order approximation of the variance may be inaccurate (Casella and Berger 2002). The latter problem is not expected to be the case when using Methods II or III for estimating the variance of the abundance estimate.

The proposed methodology is general and not necessarily limited to pup production estimation of harp or hooded seals. We expect it to work on any similar surveys that make use of georeferenced data. For abundance estimation of fish and shellfish that is based on trawl catch data, the proposed method may be applied by considering each trawl catch as a sample. The area sampled at each station is also given, since you know the speed of the vessel and the time of trawling. The method may also be used for acoustic-based abundance estimation of fish if the transect covered during a survey is divided into small sampling segments, such that a given geographical location is assigned to each segment.

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Ecological and economic considerations in the conservation and management of the Pacific sardine (*Sardinops sagax*)

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Abstract: In this paper, we consider economic and ecological issues associated with the Pacific sardine (*Sardinops sagax*) as a commercially harvested species relative to its importance as prey for species of commercial, recreational, and ecological significance. Using economic theory and ecosystem modeling results for the California Current, we investigate whether sardines might be more valuable as forage than as commercial landings. Our analysis considers various conditions under which this would be justified. For example, ex-vessel prices of commercially important sardine predators near the high end of their respective ranges in recent years and the ex-vessel price of sardine at the low end of its respective range would favor leaving more sardine as forage fish. However, even if it were advisable to reduce the volume of the sardine fishery to leave more forage for other, more valuable species, it does not necessarily imply that the sardine fishery should be shut down altogether. Rather, our results indicate that consideration of the trade-offs is a key element of ecosystem-based fishery management.

Résumé : Notre étude s'intéresse aux questions économiques et écologiques associées à la sardine du Pacifique (*Sardinops sagax*) en tant qu'espèce récoltée commercialement, relativement à son importance comme proie pour d'autres espèces qui sont d'intérêt commercial, sportif et écologique. La théorie économique et des résultats de modélisation dans le courant de Californie nous servent à déterminer si les sardines pourraient être de plus grande valeur comme fourrage que comme débarquements commerciaux. Notre analyse examine diverses conditions qui justifieraient cette assertion. Par exemple, les prix au débarquement des prédateurs de sardines d'importance commerciale dans la partie supérieure de leur gamme et les prix des sardines au débarquement dans la partie inférieure de leur gamme renforcent la proposition de laisser plus de sardines comme poissons fourrage. Cependant, même s'il est recommandable de réduire le volume de la pêche commerciale aux sardines afin de laisser plus de fourrage à d'autres espèces de plus grande valeur, cela n'entraine pas qu'il faille fermer complètement la pêche commerciale aux sardines. Nos résultats indiquent plutôt que l'examen des compromis est un des éléments essentiels de la gestion des pêches commerciales en fonction des écosystèmes.

[Traduit par la Rédaction]

Introduction

Over the last few years there has been increasing emphasis, in talks and in writing, on ecosystem management of fisheries. As Larkin (1996) recognized, ecosystem-based management "means different things to different people, but the underlying concept is as old as the hills." Larkin and others have held that the basic tenets of an ecosystem-based management approach involve a more holistic view of managing resources in the context of their environment, including the needs of other predators and dependent species in the food web (e.g., Francis et al. 2007). The fishing industry typically exploits only a fraction of all the different species of fish in the sea, and nonconsumptive uses of fish or marine mammals are concerned with still fewer species, but even so, the linkages between species need to be taken into account. The food chain is a simple metaphor useful for describing, in broad terms, what is involved, and Ryther's (1969) evaluation of food chains and energy transfer in marine ecosystems represents a very reasonable interpretation of energy transfer. Ryther's work was based on the very reasonable assumption of 10%–20% transfer efficiency between trophic levels (from primary producers such as phytoplankton to consumers such as zooplankton and fish and top carnivores such as cod, tunas, and marine mammals), and his estimates of marine production were also based on the assumption that food chains tended to be shorter in coastal upwelling ecosystems relative to continental shelves and open-ocean ecosystems.

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Ryther acknowledged that the food chain was an oversimplification of a complex system, and since his work, trophic interactions and transfer efficiencies tend to be more frequently assessed with models that take greater account of the complexity of marine food webs (although they remain gross simplifications). Among the more frequently used modeling platforms for evaluating food web interactions is Ecopath, first developed by Polovina (1984), with additional development by Christensen and Pauly (1992) and a dynamic version (Ecosim) developed by Walters et al. (2000). Ecopath is a steady-state model that emphasizes rates of production and consumption of marine populations and, as such, offers an effective and straightforward means of consolidating basic information on thermodynamics, bioenergetics, ecosystem structure, fishing mortality, and trophic interactions. Although such models are typically constrained by imperfect assumptions regarding bioenergetic rates and data limitations for most elements of the food web, the energetic accounting of the model offers an approach for quantifying both direct and indirect predator–prey interactions among commercially and ecologically important species.

Recently, attempts have been made at developing comprehensive models for ecological and economic interactions. Finnoff and Tschirhart (2003) present an approach inspired by general equilibrium economic models. In a second paper (Finnoff and Tschirhart 2004), they show how uses of an ecosystem can be evaluated economically (for an insightful and constructive criticism, see Simpson 2004). Jin et al. (2003) show how ecological and economic input–output models can be coupled to evaluate economic effects of different catch policies. Our approach is more modest; it falls somewhere between a general and a partial equilibrium model, as we look at one specific stock but consider its interactions with other stocks and base our parameters on a comprehensive ecological model.

Eichner and Tschirhart (2007) note three uses of the ecosystem: (*i*) consumptive and (*ii*) nonconsumptive uses of fish stocks and (*iii*) indirect use of a system in its natural state. Although (*i*) and (*ii*) need not be in conflict, (*i*) and (*iii*) certainly are. To resolve such conflicts, the consequences of different alternatives need to be reduced to some common denominator facilitating comparisons and trade-offs, such as monetary values. Similarly, fishing typically involves exploitation of several interrelated species, and economic considerations are unavoidable in the context of ecosystem management. Suppose we exploit two species in which one preys on the other. Should we exploit the predator heavily to limit the predation on the other stock so that we can take more of the latter? Or should we spare the prey as forage fish for the predator? The answer depends crucially on the relative prices (net of costs) of the two species. If a 90% loss is involved in the transfer of biomass from the prey to the predator, the price of the latter would have to be 10 times the price of the former to make this worthwhile. The feasibility of both would depend on sufficient food being available for the prey at the next level below.

In this paper, which builds on Field et al. (2006), we shall highlight the need for data required to implement ecosystem-based fishery conservation and management by considering the management of the Pacific sardine (*Sardinops sagax*), known to be a potentially important source of feed for other types of fish higher up in the food chain. However, quantifying how important the sardine is as a prey item depends on a number of assumptions. A complicating factor is that the relative abundance of sardine as prey has changed dramatically across time in response to the relative abundance of the sardine populations as a whole. When sardines are abundant, the logical result is that they are an important prey item for many commercially important species (including, but not limited to, Chinook salmon (*Oncorhynchus tshawytscha*), albacore tuna (*Thunnus alalunga*), and thresher sharks (*Alopias vulpinus*)), as well as ecologically important (and in some cases, endangered) predators (pinnipeds, baleen whales, and seabirds). Based on ecosystem modeling results and simple economic theory, we can ask whether it would make sense to spare the sardine to catch greater amounts of more valuable fish that prey on it, or whether it would be better to catch the sardine and sell it at the price that it normally fetches in the market.

However, a major difficulty in making such evaluations is quantifying the amount of sardine eaten by a given predator in a given period. This is particularly complicated for sardine, as low-frequency, climate-driven changes in ocean conditions have long been attributed to long-term (interdecadal) variability in reproductive success and survival in sardine, anchovy, and other coastal pelagic species that, in turn, appear to be responsible for some of the most spectacular boom and bust fisheries seen in the world's oceans (MacCall 1996; Schwartzlose et al. 1999). The ecosystem model (cf. Field et al. 2006) that we rely on here was developed for dynamic simulations of the northern California Current, starting in the 1960s but based on food habits data over a broader time period. At that time, sardines were at very low levels of abundance. As a result, the model is not likely to be representative of periods with greater sardine abundance or of predators and prey in the southern part of the California Current. Nevertheless, the relative diet proportions provide a useful starting point for exercises such as the one at hand, to highlight things we need to know better if for no other reason. To provide some guidance regarding the relative abundance of sardines in the California Current over time, especially with respect to their importance to key predators, we also include a review of major sardine predators over time (Appendix A).

Materials and methods

The value of sardines as forage fish

The size of the sardine stock is largely driven by recruitment, which varies as a result of both density-dependent (stock size) and density-independent (environmental) factors. Reducing the catches of sardine by a small amount would increase the sardine stock in the short term, with a more modest equilibrium effect on population size due to density dependence. This incremental sardine stock will die out over time, mainly through predation from other fish and marine mammals. Hence, as a first approximation, a small reduction in catch will end up as feed for other fish and marine mammals, while the effect on the equilibrium stock size will depend on the status of the population relative to its carrying capacity (with greater compensation at lower relative stock sizes). Consider, then, a reduction in the catch of sardines by $-\Delta S$, increasing the sardine stock by the same amount

ΔS. The value of this incremental sardine stock as forage would be given by

$$(1)\qquad \Delta S\sum_{i=1}^{N} P_i a_i b_i$$

where i indexes the N species that feed on the sardine, P_i is the price (net of fishing costs) of species i, a_i is the share of the incremental sardine stock that is eaten by species i, and b_i is the fraction converted to predator biomass. If ΔS is large, P, a, and b are unlikely to be constant parameters. If a large ΔS results in a large increase in the biomass of predators, the P values are likely to be affected, but in an uncertain direction; they could rise because the fish will probably become easier to catch, but they could also fall because there would be more fish on the market. The a values might be affected if there are disproportionate changes in the biomasses of individual sardine predators. The b values would be likely to fall, because a larger predator stock would need more energy for its own maintenance, converting less of its intake to weight increase. We certainly do not have anywhere near enough information to say anything about these functions; indeed, it turns out to be hard enough to put any fixed values on P, a, and b as a first approximation. Furthermore, sardine is likely to be only one of many components in the feed intake of the sardine predators and, perhaps, even a minor one, in which case constant parameters would not be a bad approximation.

If the sardine is more valuable as commercial landings than as forage fish the following must hold:

$$(2)\qquad P_s\Delta S > \Delta S\sum_{i=1}^{N} P_i b_i a_i$$

where P_s is the market price of sardine, net of fishing costs.

If we are giving up ΔS of the sardine stock permanently, we should, on the left-hand side of eq. 2, have the net present value of the permanent decrease in catches of sardines. To evaluate this in detail, we would need an intertemporal model of the sardine stock. For a moderate value of ΔS and a given discount rate, we can approximate this by dividing by the discount rate. Similarly, on the right-hand side, we would have the present value of the increase in the biomass of predators, for which we would also need a dynamic multispecies model. Again, for a moderate ΔS, a division by the discount rate would give a reasonable approximation. Because we would be dividing by the same discount rate on both sides, there is no need to consider the choice of discount rate any further, but for the later discussion, the reader should bear in mind that the prices on both sides refer to a weight unit (kilogram) on an annual basis.

The predators of the sardine are not all commercially valuable. Splitting the sum on the right-hand side of eq. 2 and assuming an average noncommercial value v per unit of biomass growth of noncommercial predators attributable to sardine consumption and canceling the ΔS on both sides gives

$$(3)\qquad P_s > \sum_{i=1}^{N} P_i b_i a_i + v\sum_{j=1}^{M} b_j a_j$$

where i is an index for the N commercially valuable species and j is an index for the M noncommercial species. The right-hand side is the forage value of the sardine, showing the value of the increase in the amount of sardine predators provided by a unit increase in the sardine stock. To be more valuable as forage species, the P_i values and the v have to be large enough to reverse the inequality. In the following, we shall calculate the critical value of v, given the P_i values observed in markets. This critical value is the value of v that turns eq. 3 into an equality.

The values of the noncommercial predators are of two types. First, there are nonextractive values associated with the presence of the stock of the predator species involved (generally, baleen whales such as humpback whales, pinnipeds such as California sea lions and fur seals, and seabirds such as the brown pelican and common murre). These values have become particularly prominent for marine mammals. In the US, these animals are no longer captured for their meat, hides, fat, or other products that they might yield, but are considered to have high existence value. Secondly, they support some nonextractive economic activities, such as whale-watching charter trips. It is unclear, however, how large the herds of these animals should be. Is it enough just to secure their continued existence or is it desirable to keep larger herds, for example, for the purpose of more opportunities of viewing these animals? Furthermore, the minimum stock size necessary for securing the continued existence of each of these predators is usually not known, and if it were, the species would already be extinct.

The other type of value from noncommercial predators is associated with recreational fishing. These are extractive values, but the market value of recreationally caught fish is likely to underestimate their recreational value, as the process of catching the fish provides satisfaction to the recreational fisherman over and above what he might get from selling his catch in the marketplace. Recreational fishermen are willing to incur considerable costs to pursue this activity, which suggests that these costs can be used as proxies for what this activity is worth. Many studies have been made over the years of what recreation is worth, and many of these employ the costs of getting to the recreational activity, the travel-cost method (on nonmarket valuation, see, e.g., van Kooten and Bulte (2000) and Grafton et al. (2004)). It could easily happen that the value of fishing as a recreational activity is worth several times what the fish could be sold for in the marketplace.

Some words of caution are in order at this point; nonmarket values such as the ones just discussed are likely to be problematic. This is particularly true of values estimated by asking people about their willingness to pay for the opportunities of viewing wild animals or of preserving the opportunity to do so at some future point in time. No one ever parts with any money for the purposes allegedly giving rise to these values. This is likely to result in an upwardly biased estimate of willingness to pay (on the upward bias that can arise in nonmarket evaluation, see Grafton et al. (2004), chapter 9 and references therein). Furthermore, this procedure can be criticized because material benefits are being compared with purely hypothetical ones that are never tested in the marketplace.

Analysis

In a model used by Field et al. (2006), the following predators of sardine appear in the diet matrix: Pacific salmon, northern albacore, coastal sharks, common murres, gulls, orcas, toothed whales, sea lions, fur seals, and baleen whales. This model was based on the northern part of the California Current; however, Leet et al. (2001) mention other predators that are important components of the southern California Current food web (for more details regarding the temporal and spatial variation in sardine predation, see Appendix A). Of the predators identified in the model by Field et al. (2006), only salmon, albacore, and coastal sharks are commercially valuable.

We shall use the data published in Field et al. (2006) to determine the parameters a and b. In their table 1, they show production divided by consumption for all species in their model. This corresponds to the parameter b, ignoring the possibility that it might, for any given predator, vary according to the prey eaten. The a values can be found in the following way:

$$(4) \qquad a_k = \frac{(g_k S_k d_k)/b_k}{mS_s}$$

The inverse of b shows the amount that the predator must eat per unit of production. The parameter g is production per unit biomass (S), and d_k is the share of sardines in the diet of predator k. The numerator thus expresses the total consumption of sardines by predator k. The parameter m is the predation mortality of sardines, so the denominator expresses how much of the sardine biomass is consumed by all predators. Table 1 in Field et al. (2006) has information on g and S, and the d values are in their table 2. (Some of the d values listed in table 2 in Field et al. (2006) are 0, due to rounding off after two decimals. In this case, the d values have been determined so as to imply consumption per biomass similar to the numbers found in table 1 in Field et al. 2006).

If the list of predators is complete, the a values must sum to one. For a given size of the sardine stock (S_s), this then determines m, the per year natural mortality of sardines (for parameters and a values, see Table 1). These values imply $m = 0.24$. Current stock assessments of sardine estimate the instantaneous natural mortality to be 0.4 (Hill et al. 2006). The instantaneous fishing mortality of sardines is close to 0.1, which implies that the natural mortality per year is 0.31. The above value $m = 0.24$ thus seems rather too low. This could be due to omission of some sardine predators, such as hake, a point that we return to below.

Results

We consider, first, the case in which fishing costs are ignored. The price of sardine and the prices (P_i values) of the commercially relevant predators of the sardine are shown (Table 1). These are average ex-vessel market prices for the period 1998–2002, expressed in year 2000 US dollars. The critical value of v (i.e., the one at which eq. 3 is an equality) turns out to be just below $7·kg^{-1} (base case in Table 2), which is to be interpreted as the value per year of a 1 kg increase in the biomass of all of the noncommercial predator species induced by the incremental supply of sardines as forage, cf. the previous discussion. Although well above the market price of predator species such as salmon and albacore, it may not be totally out of range for some

Table 1. Ecological parameters, shares (a) of sardine eaten by predators, and ex-vessel prices (P, expressed in year 2000 US dollars per kilogram) of Pacific sardine (*Sardinops sagax*) and its predators.

Predator (k)	d_k	S_k	b_k	g_k	$g_k \cdot b_k^{-1}$	a	P
Salmon (e.g., *Oncorhynchus tshawytscha*)	0.010	0.367	0.1600	0.930	5.813	0.13168	3.50
Albacore tuna (*Thunnus alalunga*)	0.050	0.014	0.0500	0.360	7.200	0.03111	1.76
Coastal sharks (e.g., *Alopias vulpinus*)	0.050	0.050	0.0600	0.180	3.000	0.04630	1.73
Common murres (*Uria aalge*)	0.001	0.009	0.0008	0.100	125.000	0.00695	
Gulls (e.g., *Larus californicus*)	0.001	0.002	0.0010	0.120	120.000	0.00148	
Orcas (*Orcinus orca*)	0.005	0.001	0.0020	0.020	10.000	0.00031	
Toothed whales (e.g., *Phocoenoides dalli*)	0.050	0.052	0.0025	0.070	28.000	0.44939	
Sea lions (e.g., *Eumetopias jubatus*)	0.010	0.012	0.0050	0.074	14.800	0.01096	
Fur seals (*Callorhinus ursinus*)	0.010	0.006	0.0025	0.091	36.400	0.01348	
Baleen whales (e.g., *Megaptera novaeangliae*)	0.090	0.075	0.0050	0.037	7.400	0.30834	
Sardine (*Sardinops sagax*)		0.663					0.10

Note: d_k, share of sardines in diet of predator k; S_k, biomass (tonnes·km^{-2}); b_k, production/consumption; g_k, production/biomass; $g_k \cdot b_k^{-1}$, consumption/biomass.

Table 2. Critical value (v, \$·kg^{-1}) of noncommercial predators that would render sardines more valuable as forage fish under different scenarios.

		Scenario					
	Base case	1	2	3	4	5	6
v	6.78	<0	0	<0	3.81	<0	16.42

Note: Scenario 1, fishing costs 75% of market price for commercial predators and 80% for sardines; scenario 2, price of sharks raised to \$8.47 per kg or price of salmon to \$4.39 or price of albacore to \$13.82; scenario 3, salmon and albacore prices 20% higher and sardine price 20% lower; scenario 4, $b = 0.2$ for salmon, albacore, and coastal sharks; scenario 5, raising d values by 40% for salmon, albacore, and coastal sharks; scenario 6, including hake as a predator.

predator species such as sharks as recreational resources or noncommercial species such as marine mammals as public goods.

To illustrate this latter point, it is possible to draw on studies of the willingness to pay for Chinook salmon and some marine mammal species. A number of such studies are summarized in Martin-Lopez et al. (2008); an earlier summary is Bulte and van Kooten (1999), which focuses on the survival of species. The willingness to pay per household for selected species listed in this overview is shown (Table 3). To convert this into total willingness to pay, we must find the appropriate number of households to include as a multiplier. It seems excessive to include all households in the US, let alone further afield, and so for illustrative purposes, we have limited ourselves to the 17.6 million households reported in the US 2007 census for the three west coast states of California, Oregon, and Washington. Dividing the total willingness to pay by the total weight of the stock produces the willingness to pay per kilogram listed (see Table 3, which lists the respective numbers and average weights for Chinook salmon (Pacific Fishery Management Council 2006, 2008) and for the selected marine mammals (Hunt et al. 2000; Barlow and Forney 2007; Carretta et al. 2007)). These numbers vary wildly and some appear unrealistically high. Note, however, that these values are once-and-for-all payments for preserving the species, whereas the \$7·kg^{-1} calculated above is on an annual basis. It is therefore the present value of this that must be compared with the willingness to pay reported by Martin-Lopez et al. (2008). This obviously depends on the discount rate chosen. At a discount rate of 10%, \$7·year^{-1} in perpetuity is worth \$70 and, at 5%, twice as much. The willingness to pay for bottlenose dolphins, Chinook salmon, and Steller sea lions far exceeds this, whereas the rest do not. Not all of the species listed prey on sardines (Table 3; grey whales and blue whales do not), but they have been included here as illustrative of nonmarket value of mammals.

There are reasons to believe that the reported willingness to pay exaggerates the nonmarket value of the species involved (Table 3). They have been derived from the willingness to pay for preserving these species, but an incremental change in the stock of these is likely to be worth much less. An increased supply of sardines is not likely to be critical for the existence of any of the predator species, but it will lead to a sustained increase in predator abundance if the increase in sardine availability does not come directly at the expense of equally substitutable forage species, such as anchovy. Indeed, it can be argued that the entire sardine stock would not be critical for the survival of any of these species, as there are many other types of forage fish on which these species feed, although such logic may not hold in the presence of large-scale fisheries for all forage fishes. Because of this or, possibly, other reasons, the increase in the stocks of noncommercial predators like marine mammals and sea birds brought about by increasing the stock of sardines might have little or no economic value.

The summary by Martin-Lopez et al. (2008) illustrates another weakness of willingness to pay studies, already mentioned, having to do with the fact that the respondents on whom these numbers are based never have to part with their money. If we assume that the same households are behind the willingness to pay for different species and sum them across species, we get more than \$2000. This is not a trivial sum to part with and might still be too low because there are many more public goods not included in the two studies that people might also be willing to pay for.

Whatever the willingness to pay for noncommercial species, it is possible to find realistic combinations of parameters that would reduce the critical value of v to or below zero, implying that a negligible value of the noncommercial predators would be enough to justify preserving the sardine as forage fish. Some of these are shown (Table 2); one case involves fishing costs. We do not have detailed information on fishing costs for sardines and the other commercially important species, but in general, v falls as the cost of fishing sardines increases or the cost of fishing the commercial predator species falls. It is quite possible to find realistic combinations of fishing costs that would produce a zero or negative value of v. This happens, for example, for fishing costs equal to 80% of the price for sardines and 75% of the price for salmon, albacore, and coastal sharks. More generally, as the net price of sardines and the commercial predators approach zero, the critical value of v approaches zero as well, i.e., it would take only an infinitesimally small value of the noncommercial predators to render the sardine more valuable as forage fish.

Another possibility is a high recreational value of sharks. If the market value of sharks were raised from \$1.73 to \$8.47·kg^{-1}, the critical value of v would be zero. In other words, if the sharks as a recreational resource are worth almost five times as much as they are worth as commercially marketed fish, this would suffice to make the sardine more valuable as forage fish than as commercially landed fish, again ignoring fishing costs. Note that in this case, the high recreational value applies to all coastal sharks, which is perhaps unlikely; although some shark species may be worth more than five times their commercial landed value as recreational resources, this does not necessarily extend to all sharks. Recreational values of the other commercial predators (salmon and albacore) would have the same effect; a moderate recreational value of salmon alone (about 25% on top of market price) would be enough to reduce the critical value of v to zero, whereas the price of albacore would have to increase almost eightfold.

Realistic changes in the market prices of the other two commercially relevant predators, as well as the sardine itself, could produce a similar result. In the 5-year period from 1998 to 2002, the highest price of salmon was 10%

Table 3. Willingness to pay (WTP; 2005 constant $) for Chinook salmon and selected marine mammals.

Species	WTP per household	No. of animals	Weight of animal (kg)	WTP·kg
Chinook salmon (*Oncorhynchus tshawytscha*)	126.66	2 200 000	7.1	142.72
Steller sea lion (*Eumetopias jubatus*)	73.83	8 350	198.0	785.95
Grey whale (*Eschrichtius robustus*)	34.70	10 000	20 000.0	3.05
Bottlenose dolphin (*Tursiops truncatus*)	23.17	3 200	188.0	677.85
Humpback whale (*Megaptera novaeangliae*)	128.34	1 400	30 000.0	53.78
Blue whale (*Balaenoptera musculus*)	44.57	1 200	103 000.0	6.35
Northern elephant seal (*Mirounga angustirostris*)	31.53	124 000	371.0	12.06

higher than the average price in the period, whereas the highest price of albacore was 20% higher than the average. The lowest price of sardine was 20% lower than the average price in 1998–2002. Raising the price of albacore and salmon by 20% from the base case and lowering the price of sardine by the same percentage reduces the critical value of v to less than zero. In other words, the value of sardine as forage fish is quite sensitive to realistic changes in its own market price and the market prices of the commercially relevant predators, and such prices as have been observed in the recent past would be sufficient to make the sardine more valuable as forage fish than as commercially landed fish.

We now consider changes in the biological parameters. These are a bit more difficult to deal with as these changes affect the a coefficients because of how they have been calibrated. Consider first a change in the value of b (the conversion efficiency) for salmon, albacore, and coastal sharks, the commercially relevant predators of sardine. The value b = 0.2 is likely to be the upper limit of this parameter. A higher value of b implies that the predators have to consume less food to produce one unit of biomass (this is given by $1/b$ in eq. 4). This will reduce the relevant a_i values if we keep the other parameters in eq. 4 unchanged, but because these coefficients sum to one if the list of predators is complete, we must adjust either m or S_s to accomplish this. The necessary adjustment in m, the natural mortality of sardine, turns out not to be very great, 0.22 instead of 0.24. Making these adjustments reduces the critical value of v below $4·kg^{-1}. Thus, a high transfer efficiency by itself would not be enough to make the sardine sufficiently valuable as forage fish for the commercially interesting predators alone, at the assumed baseline prices and ignoring fishing costs.

Changing the d parameters, the shares of sardine in the diet of the predators, also affects the a_i values, keeping the remaining parameters constant. Again the question is which other parameters in eq. 4 to change to comply with the assumption that the list of predators is complete. Raising the d values, keeping the sardine stock unchanged, would simply imply a higher natural mortality (m) of sardines; more sardines are being eaten, and with all else being equal, there must be a higher natural mortality of sardines. If the d values increase simply because there are more sardines around so that the natural mortality of sardines (m) is unchanged, the sardine stock (S_s) would increase in proportion to the d values, the a_i values would remain the same, and the conclusions drawn from eq. 3 would remain the same. Hence, the interesting problem to look at in this context are changes in the d values that would be compatible with reasonable values of the natural mortality of sardines, as such changes would reflect the uncertainty about the d values for any given level of the sardine stock.

Increasing the share of sardines in the diet of albacore, salmon, and coastal sharks by 40% would reduce v to less than zero (cf. Table 2). This produces only a moderate increase in the implied natural mortality of sardines, 0.26 instead of 0.24. These shares certainly are higher than the ones produced by the model of Field et al. (2006) but perhaps not entirely unreasonable, given the uncertainty that surrounds this parameter in any case. Note that these higher d values would pertain to the low level of the sardine stock in the 1960s, the period used to calibrate the model, and not to later years when the sardine stock had recovered and the d values presumably were higher simply because there were more sardines around. If these higher d values are realistic, it would imply that the sardine stock is more valuable as forage fish than as commercial catches due to the predation by commercially relevant species alone.

Finally, we return to the question of the low inferred natural mortality rate. This could be due to the exclusion of sardine predators. One known predator of sardines that has not been included is hake. If we add hake to the list of predators using the ecological parameter values from Table 1 of Field et al. (2006) (for hake, these are b = 0.09, g = 0.23, and S = 25.99) and calculate the d value that would produce an annual natural mortality of 0.31, consistent with an instantaneous natural mortality of 0.4 and an instantaneous fishing mortality of 0.1, we get d = 0.0007 for hake. The reason for this low value is that the biomass of hake is very large in the model, 26 tonnes·km^{-2}. This results in a much higher critical value of v than before, or more than $16·kg^{-1} (cf. Table 2). The reason is that hake has a very low ex-vessel price ($0.08·kg^{-1}), which is even lower than that for sardine, implying that from an economic perspective, it would not make sense to preserve the sardine as forage for hake. Consequently, a greater burden is put on the noncommercial species to justify preserving sardine as forage; they have to be more valuable than before.

Discussion

In this paper, we have looked at whether the Pacific sardine might be more economically valuable as forage fish than as a commercial catch. Although a definitive answer is somewhat elusive given the uncertainty in many of the parameter values, this seems quite possible. With market prices of sardine and commercial predators set at their averages for 1998–2002, the value of the increased biomass of noncommercial species would not have to be more than $\$7\cdot\text{kg}^{-1}\cdot\text{year}^{-1}$ to justify leaving the sardine in the sea as forage species. Taking fishing costs into account could reduce this critical value to zero. A recreational value of the shark, the albacore fishery, and especially the salmon fishery might be sufficient to produce this result. Furthermore, raising the prices of commercial predators and lowering the price of sardine within the range observed for the said 5-year period would be enough to tip the scale in favor of leaving the sardine as forage fish. Realistic changes in biological parameters such as transfer efficiencies and shares of sardines in the diet of predators could also make the sardine more valuable as forage fish than as commercial catches.

It must be emphasized that these results are sensitive to ecological parameters reflecting the transfer efficiency of sardine to the next trophic level, as well as the share of sardines in the diet of the different predators. The relationships that have been modeled here, with constant coefficients, are likely to be nonlinear and dependent on the relative abundance of sardines and other predators. Nonetheless, these results are consistent with a similar evaluation of the importance of forage species by Walters et al. (2005), who demonstrated that the widespread application of contemporary (MSY proxy) single-species management approaches could lead to dramatic impacts on ecosystem structure when applied to forage species. Although they did not explicitly include an economic evaluation, their results are consistent with the need to consider forage species as resources for which the total economic value is derived from both their value as a fisheries target and their role as prey to commercially and recreationally important stocks.

Even if it were advisable to reduce the volume of the sardine fishery to leave more forage for other, more valuable fish, it does not necessarily imply that the sardine fishery should be shut down altogether. The model that we have used takes predation and food conversion parameters as being fixed, but major changes in stock levels and catches are likely to affect these parameters. To confidently evaluate the effects of drastic changes such as elimination of the sardine fishery, we would need a more complete model that would take into account possible nonlinear ecological relationships. However, results from models like the present one do indicate a direction of change and illustrate that a strategic consideration of the trade-offs could be an important element of the decision-making and management process. As it happens, the current regulatory regime for Pacific sardine (as well as northern anchovy and other highly variable coastal pelagic species) explicitly considers the impact of environmental variability on productivity by varying harvest rates with a temperature-driven harvest control rule (Jacobson and MacCall 1995; Pacific Fishery Management Council 1998). This includes the application of a biomass floor below which harvest rates are forced to approach zero, a control rule that originated from the need to preserve adequate forage for seabirds and other ecologically or commercially important predators.

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Appendix A. Sardines as prey

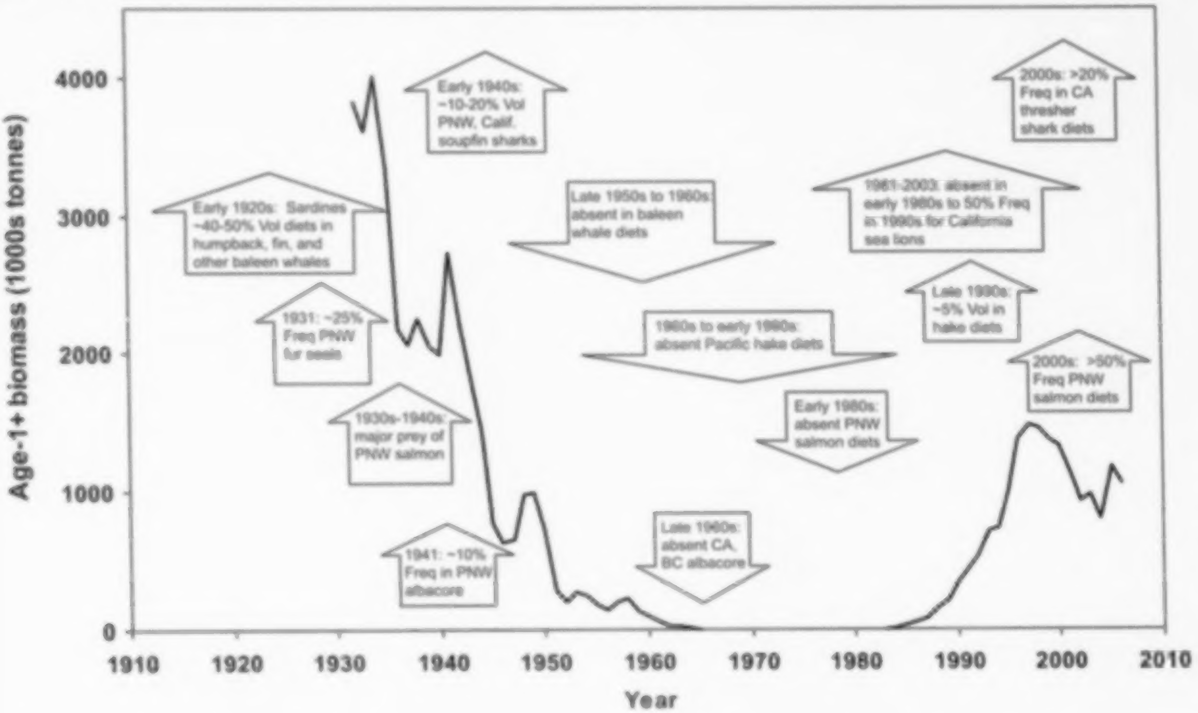
One of the key challenges in evaluating the role of sardines as forage is that this role has changed dramatically over time in response to dramatic changes in the abundance of the sardine population. In this Appendix, we present a brief summary of both historical and contemporary food habits studies over time that emphasize the often dramatic nature of these changes. Direct comparison among studies is not possible, as the studies were often limited themselves with respect to geographic region, number of animals examined, and the nature of reporting results. The latter is particularly problematic, as some studies report the frequency of occurrence of different prey items (e.g., the percentage of specimen samples in which a given prey was present), whereas others report the percentage of the total volume of prey represented by a given prey species, and still others report both or neither. Despite such inconsistencies, there is a clear pattern that reflects the importance of sardines to predators in the 1930s and 1940s, the absence of sardines in predator diets from the 1950s through the 1980s, and an increasing role of sardines as a key forage species through the 1990s into the 2000s. Several examples are shown from studies of different predators over time that illustrate this shift relative to the abundance of sardine as reported by Schwartzlose et al. (1999) for the 1930s through the 1960s and Hill et al. (2006) for the 1980s through 2006 (Fig. A1). Additional details and references for each predator in each time period are detailed below.

For Pacific salmon, Silliman (1941) evaluated Chinook and coho stomach contents off the coast of Washington in 1938 and found that Pacific sardine was an important prey item during many periods, as were other clupeids such as herring and anchovy. Pritchard and Tester (1940) found that sardines were heavily utilized by spring salmon off the west coast of Vancouver Island and in the Straight of Georgia in 1940, although they were absent in stomach contents in 1939. Chapman (1936) found that Chinook salmon off the coast of Washington in 1936 fed principally on sardines, herring, and euphausiids. By contrast, in salmon food habits studies in the early 1980s, Pacific sardines were not present, whereas clupeids such as northern anchovy and Pacific herring were among the most important prey items (Brodeur et al. 1987). However, in the early 2000s, sardines were once again noted to be key prey to Pacific Northwest salmonids, occurring in over 50% of Chinook and coho salmon sampled off the coasts of Oregon and Washington (Emmett et al. 2005).

Although relatively few historical studies of the food habits of pelagic sharks exist, Bonham (1949) reported on the food habits of 200 soupfin sharks collected off the coast of Washington in which some 16% of the total prey by volume was Pacific sardine. Similarly, Ripley (1946) reported that approximately 20% of soupfin sharks (that contained identifiable prey) collected off the coast of California between 1941 and 1944 fed on sardines. Common thresher sharks have long been described as predators of schooling coastal pelagic species such as sardine and anchovy, and Preti et al. (2004) found that sardine was present in approximately the same percentage (20%) of thresher sharks collected off the coasts of California and Oregon from 1998 to 2000. A. Preti (Southwest Fisheries Science Center, Fisheries Resources Division, 8604 La Jolla Shores Drive, La Jolla, CA 92037, personal communication, 2008) also described sardines as the most important prey to common thresher sharks between 2002 and 2005, and sardines were moderately important in the diets of mako and blue sharks during that period as well.

Early diet studies of albacore off the coasts of Washington and Vancouver Island described sardines as a moderately important prey item, as were anchovy, herring, saury, myctophids, and cephalopods (Hart 1942). However, sardines were absent from albacore diets following the collapse of the sardine population in the 1950s and 1960s (Iverson 1971). Although the limited information for bluefin tuna food habits in the California Current was also taken in the late 1960s (Pinkas 1971) and consequently does not include Pacific sardines as a prey item, bluefin are well known to be important predators of sardine in the Northwest Pacific, sufficiently so that Polovina (1996) suggests that the northern bluefin tuna change their trans-Pacific migratory patterns in response to changes in abundance of sardines in the waters off the coast of Japan.

Fig. A1. Estimated Pacific sardine biomass (solid line) relative to high (upward arrows) and low (downward arrows) estimates of sardine as in the frequency of occurrence (% Freq) or as a fraction of total volume (% Vol) in the diets of key predators over time.



Several historical food habits studies describe sardine as very important in the diets of marine mammals. Clapham et al. (1997) summarized food habits reported in over 1500 humpbacks, 169 fin whales, and 26 sei whales taken from California shore fisheries in Monterey Bay and Trinidad, California, in the 1920s, and although there was significant variability between years and locations, sardines represented approximately 50% of the total prey for humpback whales (by volume, the percentage of sardines in the diet of whales taken off Trinidad, California, was significantly less than that off Monterey Bay), 32% for fin whales, and 23% for sei whales. Food habits reported from California whaling stations in the late 1950s and early 1960s did not include sardines for any of these species (Rice 1963).

Pinnipeds have also been important predators over time. Clemens and Wilby (1933) described the contents of 25 fur seal stomachs off Vancouver Island as having a frequency of occurrence of 25% sardines, although sardines were not noted in early (and very data-poor) reports of other pinniped food habits in the California Current. However, the diets of California sea lions have been monitored off the Channel Islands since 1981 (Lowry et al. 1991; Lowry and Carretta 1999), and the frequency of occurrence of sardines in their diets has increased in synchrony with the recovery of the sardine. Sardines were not seen during the 1980s, but during the 1990s, the percentage frequency of occurrence increased from approximately 5%–10% to 40%–80% and thereafter has fluctuated with the sardine biomass (M. Lowry, Southwest Fisheries Science Center, Protected Species Division, 8604 La Jolla Shores Drive, La Jolla, CA 92037, personal communication, 2008).

Sardines have also been noted as important prey items in the diets of many species of seabirds and toothed whales, as well as warm-water game fishes off Southern and Baja California such as yellowfin (Baxter 1960), barracuda (Pinkas 1966), and other warm-water game fishes not included in the northern California Current ecosystem model. Although the lack of continuous time series of food habits for a larger suite of predators, in comparable currencies, complicates efforts to quantitatively evaluate the economic role of sardines as a forage species over space and time, it is clear that this role is dynamic and highly dependent on both the relative abundance of sardines and the relative abundance of alternative prey. Similar shifts have been observed for ecologically important prey over time in Northwest Atlantic food webs (Link and Garrison 2002; Overholtz and Link 2007). An ecosystem approach towards managing fisheries for both sardine and sardine predators will benefit from a more explicit consideration of such interactions over time, from both an economic and an ecological perspective.

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DISCUSSION / DISCUSSION

Comment on "Does the fall phytoplankton bloom control recruitment of Georges Bank haddock, *Melanogrammus aeglefinus*, through parental condition?"[1]

Mark R. Payne, Mark Dickey-Collas, and Richard D.M. Nash

Abstract: In the paper "Does the fall phytoplankton bloom control recruitment of Georges Bank haddock, *Melanogrammus aeglefinus*, through parental condition?", Friedland et al. (Can. J. Fish. Aquat. Sci. **65**(6): 1076–1086, 2008) examine a sizable number of hypotheses aiming to explain the recruitment patterns observed in Georges Bank haddock. The authors focus on a correlation between the size of the autumnal phytoplankton bloom and the survivor ratio (recruitment), concluding this to be the main factor determining recruitment, via the mechanism of adult condition at the time of spawning. Here we examine this result in close detail and re-analyse some of the data presented in the paper. We show that the recruitment metric upon which Friedland et al. base their conclusions inadvertently biases the analysis in favour of high recruitment events and against low recruitments. As a consequence, Friedland et al. disregard correlations that are, in fact, significant. Furthermore, we show that the parental condition hypothesis hinges upon a single, highly uncertain data point, without which the correlation is no longer significant. We find that evidence for the parental condition hypothesis is weak, and that in performing the analysis in the chosen manner, Friedland et al. have overlooked alternative hypotheses.

Résumé : Dans leur article « Does the fall phytoplankton bloom control recruitment of Georges Bank haddock, *Melanogrammus aeglefinus*, through parental condition? », Friedland et al. (J. can. sci. halieut. aquat. **65**(6): 1076–1086, 2008) examinent un bon nombre d'hypothèses visant à expliquer les patrons de recrutement observés chez les aiglefins du banc Georges. Les auteurs se sont intéressés particulièrement à une corrélation entre l'importance de la floraison automnale de phytoplancton et le rapport des survivants (recrutement) et en concluent qu'il s'agit là du facteur explicatif principal du recrutement, par l'intermédiaire du mécanisme de la condition des adultes au moment de la fraie. Nous examinons ce résultat en grand détail et nous analysons de nouveau certaines des données présentées dans le travail. Nous démontrons que la métrique du recrutement sur laquelle Friedland et al. basent leurs conclusions fausse par mégarde l'analyse en faveur des événements de recrutement fort et contre les recrutements faibles. En conséquence, Friedland et al. ne tiennent pas compte de corrélations qui sont, en réalité, significatives. De plus, nous montrons que l'hypothèse de la condition parentale se base sur une seule donnée très incertaine, sans laquelle la corrélation n'est plus significative. Nous trouvons donc que la preuve de l'hypothèse de la condition parentale est faible et qu'en faisant l'analyse de la manière qu'ils ont choisie, Friedland et al. négligent des hypothèses de rechange.

[Traduit par la Rédaction]

In their paper "Does the fall phytoplankton bloom control recruitment of Georges Bank haddock, *Melanogrammus aeglefinus*, through parental condition?", Friedland et al. (2008) search data sets to test the prevalent hypotheses concerning recruitment success in Georges Bank haddock and hence explain large recruitment events, with particular focus on the most recent event in 2003. They rightly warn about the use of correlations in testing environmental drivers of re-

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Fig. 1. Comparison of survivor ratio definitions. The two different survivor ratios, Friedland et al.'s $\log_{10}((R/\mathrm{SSB}) + 1)$ (dotted line) and the more standard $\log_{10}(R/\mathrm{SSB})$ (solid line), are calculated as a function of recruitment. The range of the recruitment axis covers the range observed for this stock, and the SSB is based on a value of 50 000 tonnes (approximately the observed median value).

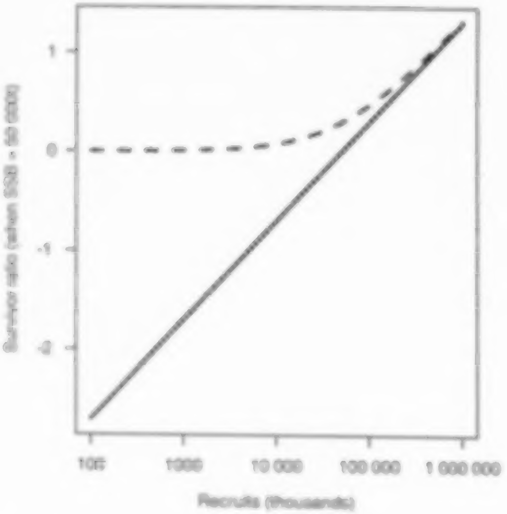
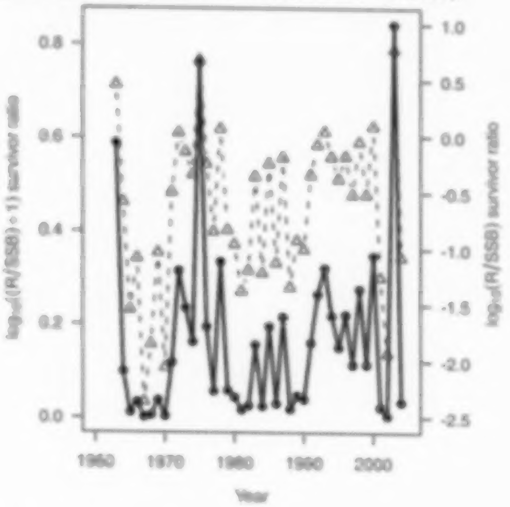


Fig. 2. Survivor ratios time series. Survivor ratio as $\log_{10}((R/\mathrm{SSB}) + 1)$ (solid circles with solid line), the definition of Friedland et al. (2008), and as $\log_{10}(R/\mathrm{SSB})$ (open triangles with dotted line) showing the difference in the relative magnitudes given to the high recruitment events. In both cases, recruits (R) is expressed in thousands and spawning stock biomass (SSB) is in tonnes. Calculations are based on stock assessment data (Brodziak et al. 2006).



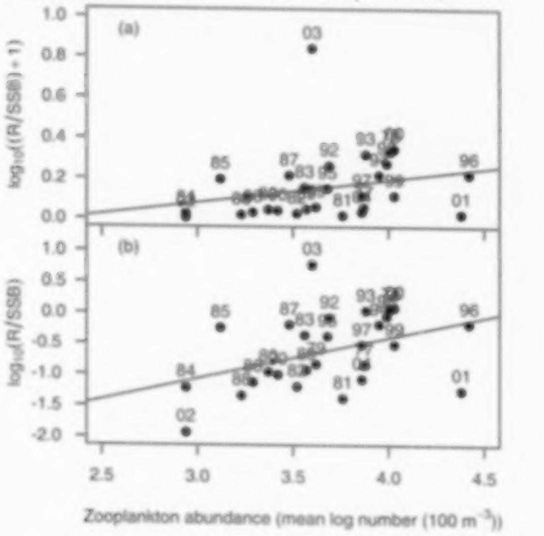
cruitment processes and then proceed to present an analysis based on a series of such correlations. As a result of one significant correlation, Friedland et al. conclude that the only plausible hypothesis is that the autumn phytoplankton bloom has a causal effect on recruitment. They propose that increased autumn chlorophyll *a* abundance results in increased flux of organic matter to the benthos, increasing prey availability and thereby improving parental condition. They subsequently infer higher egg quality from improved condition, leading to greater probability of larval survival and therefore elevated recruitment. They conclude by challenging the academic community to undertake more thorough analyses of this and alternative hypotheses.

The analysis of Friedland et al. is based around examining correlations between certain key parameters and the "survivor ratio". This commonly employed metric characterizes the magnitude of recruitment (R) while accounting for the effect of the spawning stock biomass (SSB) (Rothschild and Fogarty 1998; Liermann and Hilborn 2001) and is normally applied under the assumption of a lognormal error structure, i.e., as $\log_{10}(R/\mathrm{SSB})$ (Myers et al. 1999; Minto et al. 2008; Möllmann et al. 2008). However, the implementation employed by Friedland et al. not correctly described in the paper but confirmed by the authors to us (K.D. Friedland, NOAA, National Marine Fisheries Service, Narragansett, RI 02882, USA, personal communication, 2008) was $\log_{10}((R/\mathrm{SSB}) + 1)$, subtly but vitally different from the standard approach.

Although it may seem a small point, this choice of metric is crucial for the outcome of the paper. Firstly, the addition of the arbitrary constant 1 distorts the relationship between the recruits per spawner ratio, R/SSB, and their definition of the survivor ratio, $\log_{10}((R/\mathrm{SSB}) + 1)$, reducing the relative magnitude of low recruitment events and compressing them into a very narrow dynamic range close to zero (Fig. 1). By contrast, the $\log_{10}(R/\mathrm{SSB})$ metric gives an equal response to all recruitment events. As a consequence, we find that the metric of Friedland et al. gives the observed high recruitment events much greater relative weighting, while downweighting the low recruitment events (Fig. 2), behaving in a very similar way to the untransformed ratio. Such a change has implications for the outcomes of the analyses performed. As an example, fig. 4*b* of Friedland et al., which examines the relationship between the abundance of zooplankton and the survivor ratio, was reconstructed by digitizing the printed version of the figure to recover the zooplankton data and calculating the survivor ratios based on the original stock assessment (Brodziak et al. 2006). In this manner, we were able to reproduce the correlation analysis of Friedland et al. to an acceptable accuracy (e.g., $r_{\text{Friedland et al.}} = 0.246$, $r_{\text{Reconstructed here}} = 0.247$) and confirm the absence of a significant correlation between Friedland et al.'s survivor ratio and the zooplankton abundance (Fig. 3*a*; $p = 0.215$). However, when the more standard definition of the survivor ratio was used in this analysis, a significant correlation was indeed found (Fig. 3*b*; $p = 0.031$). We therefore conclude that the analysis method employed by Friedland et al. has a significant bearing on the results and that their data, if analysed in the more standard manner, suggest that mechanisms other than the proposed parental condition hypothesis are also consistent with observations.

The key result in this paper is the correlation between parental condition and the survivor ratio (fig. 9 in Friedland et al. 2008). Digitizing these data as above, it was possible to recalculate the correlation using the more standard definition of the survivor ratio, $\log_{10}(R/\mathrm{SSB})$: the correlation still holds

Fig. 3. Re-analysis of Fig. 4*b* from Friedland et al. (2008) showing the relationship between haddock survivor ratio and the March–April abundance of *Pseudocalanus* spp. on Georges Bank for the years after spawning. Data points are labelled with two-digit years. (*a*) Data as presented in Friedland et al. (2008) using the $\log_{10}((R/SSB) + 1)$ survivor ratio. Correlation is not significant ($r = 0.247$, df = 25, $p = 0.215$) but agrees very closely with the analysis of Friedland et al., suggesting appropriate digitization. (*b*) The same abundance data analysed using the $\log_{10}(R/SSB)$ survivor ratio. Correlation is significant ($r = 0.416$, df = 25, $p = 0.031$).



($r^2 = 0.764$, df = 5, $p = 0.010$), although it is appreciably weakened. However, as in the original analysis of Friedland et al., the correlation is driven largely by a single extreme point (the 2003 year class). The leverage of this point is extremely high, nearly 2.5 times the average value for the regression, confirming the strong influence and suggesting that the point deserves close attention (Davison 2003). The recruitment estimates used in this analysis are derived from a tuned virtual population analysis (VPA), with 2004 as the terminal year. The 2003 estimate will therefore be based on a very limited number of observations of the year class, and the uncertainty associated with this specific estimate will therefore be high. Omitting 2003 from the correlation, as a test of its influence, causes the correlation to pass outside the 95% confidence interval ($r^2 = 0.620$, df = 4, $p = 0.063$). It therefore appears that the main conclusion of Friedland et al., when re-analysed using the standard definition of the survivor ratio, is driven primarily by this highly uncertain data point.

Although we recognise that large recruitments are often "rare events", and thus analysis can be difficult, inferring a relationship on the basis of a single uncertain data point in a short time series would seem prone to error. The time series of SeaWiFS chlorophyll data used in the analysis is very short (7 years) and only contains a single "high recruitment" event: longer time series should be explored to either test hypotheses or provide more than one extreme event. Alternative time-resolved indices of chlorophyll abundance in this region spanning much longer and different time frames do exist, e.g., the CPR phytoplankton colour index (PCI) for the Georges Bank region (1961–2006, with intermittent breaks in coverage) and the Coastal Zone Colour Scanner (CZCS) satellite sensor (1976–1986). These data sets are readily available in the public domain and could easily have been added to the analysis to further examine the parental condition hypothesis.

The described correlation between the survivor ratio and autumn chlorophyll *a* abundance is also unusually high (r^2 of 0.966 for Friedland et al.'s analysis, 0.764 by the standard analysis method); indeed, this is what drew our attention to the work. However, the mechanism that the authors employ to explain this result involves a long sequence of independent steps, each of which one would expect to be highly variable in nature. That such a tight correlation is observed in the event of there being an actual causal link would seem unlikely: the natural variability in the links between each process would need to be unrealistically small for that to be the case. The time series analysed is also quite short in length (seven points), raising the very real possibility of a spurious correlation. Alternatively, the covarying autumn chlorophyll *a* abundance and recruitment may both be consequences of a third, unseen, mechanism (e.g., warmer water temperatures), a case of correlation but not causality. Use of longer time series (such as those mentioned above), together with a detailed examination of each step in the proposed mechanism, will help to clarify the nature of the correlation.

Our re-analysis of the data presented in the paper shows that at least one of the mechanisms dismissed by Friedland et al. (that of the link between the March–April abundance of zooplankton and the survivor ratio) is, in fact, consistent with the observations. Given the much simpler and more direct nature of this mechanism (increased zooplankton abundance increases food for the juveniles and thereby increases survival), it could be argued via Occam's razor that the zooplankton hypothesis is also more plausible than the parental condition hypothesis.

Although we recognise the authors' speculative intention in trying to present new hypotheses and test established ones, we have accepted the challenge laid down and have come to different conclusions. Their investigation is based on a recruitment metric that inadvertently biases the analysis in favour of high recruitment events and against low recruitments: analyses based on a standard definition of the survivor ratio and using the same source data weaken the case for the parental condition hypothesis, showing it to be driven solely by a single, highly uncertain, outlier. Furthermore, re-analysis of a subset of the presented data also suggests that alternative mechanisms (e.g., zooplankton abundance) are also plausible. We are therefore forced to conclude that the parental condition hypothesis raised in this paper is only weakly supported by the data that it contains, and that due to the analysis method employed, Friedland et al. have overlooked alternative hypotheses that may also explain the observed recruitment fluctuations.

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DISCUSSION / DISCUSSION

Reply to the comment by Payne et al. on "Does the fall phytoplankton bloom control recruitment of Georges Bank haddock, *Melanogrammus aeglefinus*, through parental condition?"[1]

Kevin D. Friedland, Jonathan A. Hare, Grayson B. Wood, Laurel A. Col, Lawrence J. Buckley, David G. Mountain, Joseph Kane, Jon Brodziak, R. Gregory Lough, and Cynthia H. Pilskaln

Abstract: Payne et al. (Can. J. Fish. Aquat. Sci. **66**: 869–872, 2009) raised several points concerning the handling and interpretation of data that went into an analysis of the population dynamics of Georges Bank haddock that suggested a relationship between the fall phytoplankton bloom and recruitment (Can. J. Fish. Aquat. Sci. **65**: 1076–1086, 2008). Their main points were the manner in which logarithmic transforms were applied, whether the 2003 year class was truly as large as estimated in a 2006 assessment, and if correlation analyses of zooplankton data should be reconsidered. The reply to these comments was aided by a new assessment which provided additional years of data and improved the quality of the recruitment time series. The reply analyses showed that the relationships were robust to the way the logarithmic transform was applied, the initial estimates of the size of the 2003 year class were correct, and relationships between recruitment and spring zooplankton biomass levels remain statistically insignificant. From these new analyses, the interpretations and conclusions reached in the original paper remain the same; the fall bloom has emerged as a candidate explanatory variable for the stock independent variation in haddock recruitment on Georges Bank.

Résumé : Payne et al. (J. can. sci. halieut. aquat. **66**: 869–872, 2009) ont soulevé plusieurs questions concernant la manipulation et l'interprétation des données utilisées dans une analyse de la dynamique de population des aiglefins du banc Georges qui a proposé l'existence d'une relation entre la floraison automnale de phytoplancton et le recrutement (J. can. sci. halieut. aquat. **65**: 1076–1086, 2008). Leurs interrogations principales portaient sur la façon dont les transformations logarithmiques ont été faites, sur la classe annuelle de 2003 à savoir si elle était vraiment aussi importante que l'indique une évaluation de 2006, ainsi que sur la pertinence de revoir les analyses de corrélation des données de zooplancton. Notre réplique à ces commentaires a été facilitée par une nouvelle évaluation qui a ajouté des années supplémentaires de données et amélioré la qualité de la série chronologique de données de recrutement. Les analyses faites pour notre réplique montrent que les relations sont robustes en regard de la façon dont les transformations logarithmiques ont été réalisées, que les évaluations initiales de la taille de la classe annuelle de 2003 sont exactes et que les relations entre le recrutement et les niveaux de biomasse printanière du zooplancton demeurent statistiquement non significatives. Suite à ces nouvelles analy-

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ses, les interprétations et les conclusions de l'article original restent les mêmes; la floraison automne ressort comme variable explicative de choix de la variation indépendante du stock dans le recrutement des aiglefins sur le banc Georges.

[Traduit par la Rédaction]

In their comment on "Does the fall phytoplankton bloom control recruitment of Georges Bank haddock, *Melanogrammus aeglefinus*, through parental condition?", Payne et al. (2009) raise a number of points concerning methodology and data interpretation. Principal among these was (*i*) transformation of the stock–recruitment data, (*ii*) strength of the 2003 year class and use of recruitment data from the end of an assessment time series, and (*iii*) whether a relationship between recruitment and zooplankton abundance deemed insignificant in the original paper was in fact significant. In the context of the original paper, these were valid concerns and represent a productive scientific exchange that is useful in our collective efforts to understand fish recruitment processes. In this reply, we will reexamine the three issues raised by Payne et al. (2009). However, there is one point we would like to make clear at the outset: we feel that Payne et al. (2009) misrepresented our conclusions related to haddock recruitment. They characterize our review of existing recruitment hypotheses and the evidence that we present for the parental condition hypothesis as "concluding this to be the main factor determining recruitment". We were cognizant of the small sample size of the data supporting the parental condition hypothesis, and contrary to their characterization, we carefully avoided the sort of overstatement they attribute to our paper. Our main conclusion was and remains "… synoptic data on the fall bloom are limited and our analyses are correlative, our purpose is to stimulate a rigorous test of this promising [hypothesis]". Our reexamination of data related to the issues raised by Payne et al. (2009) has not changed our original conclusion.

As is the case with most commercially important fish stocks, assessment of Georges Bank haddock is an ongoing process. Hence, this reply benefits from a recent assessment update of the Georges Bank stock (Northeast Fisheries Science Center (NEFSC) 2008), allowing us to address the issues raised by Payne et al. (2009) with new and updated stock–recruitment data and additional satellite and field observations.

Issue (*i*) from Payne et al. (2009) was concerned with transformation of the recruit per spawner data. Payne et al. point out that contemporary use of logarithmic transformations for recruit per spawner (*R*/SSB) indices are applied without the addition of 1 to the ratio (Minto et al. 2008; Möllmann et al. 2008). We took it as routine to add 1 before taking the logarithm (Sokal and Rohlf 1981); however, we acknowledge the concern about the transformation and have retested the relationships of interest using both transformations.

Issue (*ii*) was related to the strength of the 2003 year class. The main finding of our paper is the correlation between *R*/SSB and the magnitude of the fall bloom (fig. 9 in Friedland et al. 2008). Concerns raised by Payne et al. (2009) included the use of logarithmic transformation, but of greater importance, they suggested that the data characterizing the 2003 year class should not be included in the analysis because it was near the end of the assessment time series and was likely not well estimated. With the new assessment of the Georges Bank haddock stock, the terminal year of the assessment is now 2007, thus the 2003 year class is well estimated. In addition, this year class has been characterized by multiple fishery-independent surveys and is known to be large. We included recruitment data to 2006, considering the 2007 recruitment to be inadequately estimated by the current assessment.

The recruits per spawner ratio, based on the updated stock–recruitment data, is highly correlated to the magnitude of the fall bloom for both variants of the logarithmic transformation (Fig. 1; Table 1). The correlation using the logarithmic transformations of $(R/\mathrm{SSB}) + 1$ was slightly higher, but both variants suggest the same interpretation. Though not a point raised by Payne et al. (2009), we did not include an assessment of the potential effect of autocorrelation in our original analysis. As time series data, it is likely that these observations are not independent. Using the differencing approach suggested by Pyper and Peterman (1998), we found that the correlations using the new assessment data and both variants of the logarithmic transformation remained significant after correcting for autocorrelation (Fig. 2; Table 1). As in our original paper, the most compelling aspect of this relationship is that the fall bloom data offer an explanation for the 2003 recruitment event.

Issue (*iii*) from Payne et al. (2009) questioned the relationship between *R*/SSB and the abundance of the zooplankton *Pseudocalanus* spp. (March–April) (fig. 4*b* in Friedland et al. 2008). In addition to applying the new assessment data and testing the sensitivity of the relationships to the logarithmic transformations, we expanded the analysis to include an examination of the effect of total Copepoda abundance. In Friedland et al. (2008), we restricted our analysis to the effect of spring abundance of *Pseudocalanus* spp. based on Buckley and Durbin (2006), who found significant correlations between haddock larval growth and *Pseudocalanus* spp. abundance and only marginally significant correlations between growth and other copepods. We now include a test of correlation between the *R*/SSB and the total Copepoda abundance on Georges Bank for three time periods: January–February, March–April, and May–June (Figs. 3*a*, 3*c*, and 3*e*, respectively). These relationships were all nonsignificant, yet we note that correlations for the March–April period were only marginally nonsignificant, pointing to the importance of food availability (Table 1). When the analysis for the relationship between *R*/SSB and *Pseudocalanus* spp. abundance are repeated, we find similar results (Figs. 3*b*, 3*d*, and 3*f*). With these data, the effect of the variants of logarithmic transformation had mixed results; in some cases, one variant produced higher correlation, and in others, the opposite. We do not dismiss the contribution of adequate feeding opportunity for larval haddock to the recruitment process, nor do we discount the importance of

Table 1. Linear relationships between survivor ratio and chlorophyll and zooplankton production indices.

Recruitment index	Production index	*R*	*N*	*P*
Survivor ratio, $\log(x + 1)$	Chlorophyll *a* magnitude	0.972	9	**<0.001**
Survivor ratio, $\log(x)$	Chlorophyll *a* magnitude	0.883	9	**0.002**
Differenced survivor ratio, $\log(x + 1)$	Differenced chlorophyll *a* magnitude	0.985	8	**<0.001**
Differenced survivor ratio, $\log(x)$	Differenced chlorophyll *a* magnitude	0.929	8	**0.001**
Survivor ratio, $\log(x + 1)$	Total Copepoda (Jan.–Feb.)	0.051	26	0.806
Survivor ratio, $\log(x)$	Total Copepoda (Jan.–Feb.)	0.206	26	0.312
Survivor ratio, $\log(x + 1)$	Total *Pseudocalanus* spp. (Jan.–Feb.)	–0.031	26	0.880
Survivor ratio, $\log(x)$	Total *Pseudocalanus* spp. (Jan.–Feb.)	0.186	26	0.362
Survivor ratio, $\log(x + 1)$	Total Copepoda (Mar.–Apr.)	0.343	29	0.069
Survivor ratio, $\log(x)$	Total Copepoda (Mar.–Apr.)	0.311	29	0.100
Survivor ratio, $\log(x + 1)$	Total *Pseudocalanus* spp. (Ma.r–Apr.)	0.246	29	0.198
Survivor ratio, $\log(x)$	Total *Pseudocalanus* spp. (Mar.–Apr.)	0.359	29	0.056
Survivor ratio, $\log(x + 1)$	Total Copepoda (May–June)	–0.060	26	0.771
Survivor ratio, $\log(x)$	Total Copepoda (May–June)	–0.039	26	0.849
Survivor ratio, $\log(x + 1)$	Total *Pseudocalanus* spp. (May–June)	–0.021	26	0.921
Survivor ratio, $\log(x)$	Total *Pseudocalanus* spp. (May–June)	0.016	26	0.939

Note: Survivor ratio is transformed with $\log(x + 1)$ and $\log(x)$, where x represents recruits·SSB^{-1}. *R* is the correlation coefficient, *N* is the sample size, and *P* is the probability level associated with *R*.

Fig. 1. Survivor ratio (recruits·SSB^{-1}) for the Georges Bank haddock stock versus chlorophyll magnitude of the fall bloom on Georges Bank. Survivor ratio is transformed with log((recruits·SSB^{-1}) + 1), indicated by the solid line and solid circles, and with log(recruits·SSB^{-1}), indicated by the shaded line and shaded circles.

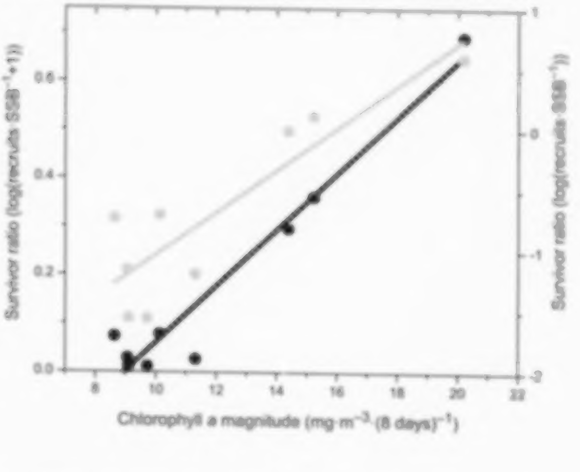
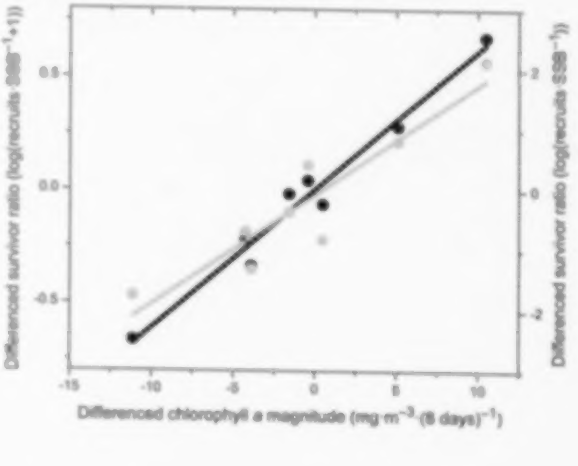


Fig. 2. Differenced survivor ratio (recruits·SSB^{-1}) for the Georges Bank haddock stock versus differenced chlorophyll magnitude of the fall bloom on Georges Bank. Survivor ratio is transformed with log((recruits·SSB^{-1}) + 1), indicated by the solid line and solid circles, and with log(recruits·SSB^{-1}), indicated by the shaded line and shaded circles.

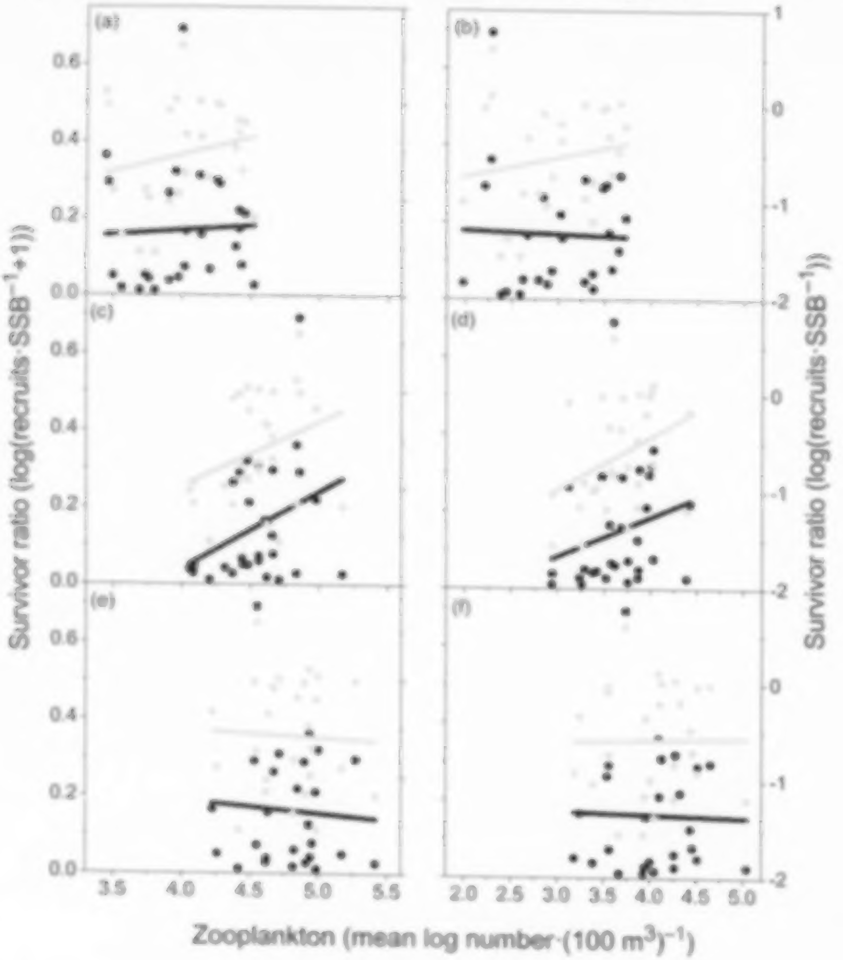


other factors such as favorable retention conditions or juvenile growth. However, for a series of recruitments of the Georges Bank haddock stock that displayed a sizeable range in survivor ratio, including the exceptional recruitment that occurred in 2003, the effect of fall bloom conditions emerged as a strong explanatory variable. Other well-studied hypotheses fall short in this regard, especially in the context of the 2003 year class.

Payne et al. (2009) expressed the opinion that other sources of data could have been added to the analysis to extend the length of the fall bloom magnitude time series. Specifically, they suggest the CPR phytoplankton color index (PCI) for the Georges Bank region and the Coastal Zone Color Scanner (CZCS) satellite sensor remote sensing data as candidate sources. We were aware of these data and analyzed them with the intent of including them in the analysis. Our conclusion after working with the data was that we could not develop compatible data to the contemporary SeaWiFS time series. We have suspended these efforts, but we are aware of plans to reprocess the CZCS data to make it comparable to the SeaWiFS data. We will revisit this issue if the data are reprocessed in a satisfactory fashion.

After consideration of the concerns raised by Payne et al. (2009), our conclusions remain unchanged: of the range of hypotheses that have been tested over the years to explain the variability in recruitment success of haddock on Georges Bank, none appears to provide a definitive explanation of

Fig. 3. Survivor ratio (recruits·SSB^{-1}) for the Georges Bank haddock stock versus spring zooplankton abundance on Georges Bank. Survivor ratio is transformed with log(recruits·SSB^{-1})+1), indicated by solid lines and solid circles, and with log(recruits·SSB^{-1}) indicated by shaded lines and shaded circles. Data for total Copepoda abundance during (*a*) January–February, (*c*) March–April, and (*e*) May–June. Data for total *Pseudocalanus* spp. abundance during (*b*) January–February, (*d*) March-April, and (*f*) May–June.



this variability. Our explorative analysis into the potential role of the fall bloom and its effect on condition of overwintering prespawning haddock is no more or less well supported then when we first suggested it. It is an idea based on a short time series of data, lacking ancillary data to test it further. However, it is not without merit as it provides a test of the hypothesis and critically offers an explanation for extreme recruitment events. As we stressed in the original paper, the greater issue is to insure that the idea is tested further.

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Contents (concluded) / Table des matières (suite et fin)

Daisuke Goto and William G. Wallace 836–846 Influences of prey- and predator-dependent processes on cadmium and methylmercury trophic transfer to mummichogs (*Fundulus heteroclitus*)

Arnt-Børre Salberg, Tor Arne Øigård, Garry B. Stenson, Tore Haug, and Kjell T. Nilssen 847–858 Estimation of seal pup production from aerial surveys using generalized additive models

Rögnvaldur Hannesson, Samuel Herrick, Jr., and John Field 859–868 Ecological and economic considerations in the conservation and management of the Pacific sardine (*Sardinops sagax*)

DISCUSSION DISCUSSION

Mark R. Payne, Mark Dickey-Collas, and Richard D.M. Nash 869–872 Comment on ''Does the fall phytoplankton bloom control recruitment of Georges Bank haddock, *Melanogrammus aeglefinus*, through parental condition?''

Kevin D. Friedland, Jonathan A. Hare, Grayson B. Wood, Laurel A. Col, Lawrence J. Buckley, David G. Mountain, Joseph Kane, Jon Brodziak, R. Gregory Lough, and Cynthia H. Pilskaln 873–877 Reply to the comment by Payne et al. on ''Does the fall phytoplankton bloom control recruitment of Georges Bank haddock, *Melanogrammus aeglefinus*, through parental condition?''

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RAPID COMMUNICATION / COMMUNICATION RAPIDE

Cindy J. Smith, Bret S. Danilowicz, and Wim G. Meijer 713–716 Bacteria associated with the mucus layer of *Merlangius merlangus* (whiting) as biological tags to determine harvest location

ARTICLES / ARTICLES

Anni Tonteri, Alexei Je. Veselov, Alexander V. Zubchenko, Jaakko Lumme, and Craig R. Primmer 717–735 Microsatellites reveal clear genetic boundaries among Atlantic salmon (*Salmo salar*) populations from the Barents and White seas, northwest Russia

David W. Welch, Michael C. Melnychuk, Erin R. Rechisky, Aswea D. Porter, Melinda C. Jacobs, Adrian Ladouceur, R. Scott McKinley, and George D. Jackson 736–750 Freshwater and marine migration and survival of endangered Cultus Lake sockeye salmon (*Oncorhynchus nerka*) smolts using POST, a large-scale acoustic telemetry array

Deborah R. Hart and Antonie S. Chute 751–758 Verification of Atlantic sea scallop (*Placopecten magellanicus*) shell growth rings by tracking cohorts in fishery closed areas

Hjálmar Hátún, Mark R. Payne, and Jan Arge Jacobsen 759–770 The North Atlantic subpolar gyre regulates the spawning distribution of blue whiting (*Micromesistius poutassou*)

Hiroshi Okamura and Yasuko Semba 771–780 A novel statistical method for validating the periodicity of vertebral growth band formation in elasmobranch fishes

Barry A. Berejikian, Donald M. Van Doornik, Julie A. Scheurer, and Richard Bush 781–789 Reproductive behavior and relative reproductive success of natural- and hatchery-origin Hood Canal summer chum salmon (*Oncorhynchus keta*)

Frederick S. Scharf, Jeffrey A. Buckel, and Francis Juanes 790–801 Contrasting patterns of resource utilization between juvenile estuarine predators: the influence of relative prey size and foraging ability on the ontogeny of piscivory

Casey Justice, Brian J. Pyper, Raymond C.P. Beamesderfer, Vaughn L. Paragamian, Pete J. Rust, Matthew D. Neufeld, and Susan C. Ireland 802–815 Evidence of density- and size-dependent mortality in hatchery-reared juvenile white sturgeon (*Acipenser transmontanus*) in the Kootenai River

Richard P. Barbiero, Mary Balcer, David C. Rockwell, and Marc L. Tuchman 816–828 Recent shifts in the crustacean zooplankton community of Lake Huron

G.J. Piet and F.J. Quirijns 829–835 The importance of scale for fishing impact estimations

Continued on inside back cover / Suite au verso

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